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Life Systems, Inc., Cleveland, OH

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This document summarizes the health effects of Benzene. Topics include: General Information and Properties; Human Exposure; Pharmacokinetics; Health Effects in Animals and Humans; Mechanisms of Toxicity; Risk Assessment and Quantification of Toxicological Effects.

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*Prepared by the Science and Technology Branch

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I. SUMMARY

Benzene is one of the world's major commodity chemicals. It is derived from petroleum and coal and is used both as a solvent and as a starting material in chemical syntheses. The numerous industrial uses of benzene over the last century need not be recounted here, but the most recent addition to the list of uses of benzene is as a component in a mixture of aromatic compounds added to gasoline for the purpose of replacing lead compounds as anti-knock ingredients.

The best known and longest recognized toxic effect of benzene is the depression of bone marrow function seen in occupationally exposed individuals. These people have been found to display anemia, leucopenia, and/or thrombocytopenia. When pancytopenia, i.e., the simultaneous depression of all three cell types, occurs and is accompanied by bone marrow necrosis, the syndrome is called aplastic anemia. In addition to observing this disease in humans and relating it to benzene exposure, it has been possible to establish animal models which mimic the human disease. The result has been considerable scientific investigation into the mechanism of benzene toxicity.

Although the association between benzene exposure and aplastic anemia has been recognized and accepted throughout most of this century, it is only recently that leukemia, particulary of the acute myelogenous type, has been related to benzene.

The acceptance of benzene as an etiological agent in aplastic anemia in large measure derives from our ability to reproduce the disease in most animals treated with sufficiently high doses of benzene over the necessary time period. Unfortunately, despite extensive efforts in several laboratories, it has not been possible to establish a reproducible, reliable model for the study of benzene-induced leukemia. The recent demonstration that several animals exposed to benzene either by inhalation or in the drinking water during studies by Drs. B. Goldstein and C. Maltoni (cf. section VI) suggests that such a model may be forthcoming. Nevertheless, at this time it is not clear whether bone marrow damage of the type that leads to aplastic anemia is required for the development of leukemia.

Most studies of benzene toxicity have involved dosing animals with benzene either by inhalation or by injection using high doses to ensure a toxic reponse. Very few studies have concentrated on the oral route of administration and none have concentrated on administering benzene by mouth at the low doses occasionally detected in drinking water. Thus, the evaluation of benzene toxicity in this report takes advantage of the benzene literature as it currently exists and cannot directly answer the questions posed by the problem of benzene in drinking water, although it is known that benzene can be absorbed via the GI tract. Nevertheless there has not been a demonstration showing bone marrow depression by benzene can be avoided by selecting an alternate route of administration.

The report will summarize the exposure of the population to benzene with emphasis on exposure through water. The toxicology of benzene in animals will be reviewed and the problem of attempts to develop an animal model for benzene-induced leukemia will be covered. Emphasis will be given to a discussion of modern theories on the mechanism of benzene induced toxicity and the role played by benzene metabolism. A summary of studies of potential mutagenicity and teratogenicity is included. The extensive literature on both the toxicity and carcinogenicity effects of benzene in humans is described. Finally, a section on carcinogenic risk assessment has been prepared.

II. GENERAL INFORMATION AND PROPERTIES

A. Physical and Chemical Properties

Benzene is an aromatic hydrocarbon, has the molecular formula C_6H_6 and a molecular weight of 78.1 (Weast, *et al.*, 1965). Under standard conditions, benzene is a colorless liquid with a very characteristic odor. It is highly flammable (limits of flammability in air of 1.5-8.0% by volume, and flashpoint of -11.1°C) and is volatile (vapor pressure of 100 mm Hg at 26°C). Benzene is relatively soluble in water (1.8 g/L at 25°C) and miscible with a variety of organic solvents. Its density, 0.8737 g/mL at 25°C, is lower than that of water so that undissolved benzene floats on top of water. The pure liquid freezes at 5.553°C and boils at 80.100°C (Ayers and Muder, 1964). The vapors of benzene are nearly three times heavier than air (Lange and Forker, 1961), causing them to settle in low places if the ambient air is relatively still.

Benzene forms a two-phase, minimum boiling azeotrope with water at a benzene concentration of 91% by weight, boiling at 69°C. It also forms ternary azeotropes with other organic compounds and water (Perry and Chilton, 1973; Lange and Forker, 1961; Horsely, 1974). This factor must be considered if evaporative purification systems are used to remove benzene from water.

B. Manufacturing and Uses

Benzene is produced in huge quantities in the U.S. A total of 1488 million gallons of industrial and specification grades were produced in the U.S. in 1978, 96% of which came from petroleum refining operations (1435 million gallons) and the remaining 4% of which were derived from coke oven operations (53 million gallons). The contributions from coke oven operations has diminished markedly over an 11-year period: 91 million gallons in 1967; 65 million gallons in 1977; and 53 million gallons in 1978 (U.S. ITC, 1979). The U.S. ITC (1979) listed 32 manufacturers as petroleum refining sources in 1978.

A very large portion of benzene is also a component in gasoline (average concentration < 1% (Runion, 1975)). It is important, particularly for the unleaded fuels, because of its antiknock characteristics. For the past several years prior to and including 1978, about 1650 million gallons of benzene were used in gasoline (U.S. ITC, 1979). For the same period, most of the industrial and specification grade benzene, approximately 1400 million gallons, were produced for chemical conversion, such as the manufacture of ethylbenzene/styrene (in polystyrene plastics), cyclohexane (in nylon), cumene/phenol (in phenolic resins for construction, automobiles, appliances and numerous other uses) and aniline (for urethanes and urethane elastomers) (U.S. ITC, 1979). It is also a preliminary raw material for chemicals such as nitrobenzene, maleic anhydride, chlorobenzenes, detergent alkylate (Mara and Lee, 1978) and pesticides (OSHA, 1978).

A much smaller amount, e.g., < 2% (Mara and Lee, 1978) is used for solvent purposes in such products as trade and industrial paints, rubber cements, adhesives, paint removers, in the artificial leather, rubber goods and rotogravure industries, and as a laboratory solvent (OSHA, 1979; Mara and Lee, 1978).

C. Extent and Significance of Problem

One study estimated that 79 million pounds of benzene are lost annually during commercial production, storage and transport (Walker, 1976). This is largely from petroleum cracking facilities, but also from coke oven operations. These emissions are related to operations such as equipment repair, the cleanup of small spills and transfer of materials. Process equipment such as blow down systems, wastewater separators, cooling towers and storage facilities, and leaking production components and seals contribute fugitive emissions as well. Operations with gasoline (storage, transport and use) also contribute to the total annual loss. Projections of emission from such sources have been reported in detail by Mara and Lee (1978) and OSHA (1978).

Due to benzene's volatility and solubility, it would be likely to migrate easily through the environment. In addition to direct contamination of water, a certain proportion of the benzene in the atmosphere will partition into water droplets (e.g., in clouds) and enter surface waters as precipitation. Unsubstituted benzene (chemically unmodified) does not react with water, except at elevated temperature and pressure. Therefore, hydrolysis is unlikely. Microbial degradation of benzene has been observed during wastewater treatment, indicating that biodegradation probably occurs naturally--though probably very slowly (Mara and Lee, 1978). Based on their review of various laboratory experiments with ultraviolet light, Mara and Lee (1978) have concluded that atmospheric degradation by photochemical reactions is also possible under certain conditions. Limited absorption of benzene by naturally occurring clays and humus may also occur. In view of the heavy environmental emissions of benzene and the limited natural removal processes, its widespread presence in the environment is likely, however.

III. HUMAN EXPOSURE

Humans may be exposed to benzene in drinking water, food, and air. Detailed information concerning the occurrence of and exposure to benzene in the environment is presented in another document entitled "Occurrence of Benzene in Drinking Water, Food, and Air" (Letkiewicz et al. 1983). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

Exposure Estimation

This analysis is limited to drinking water, food, and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to benzene from sources other than the three considered here, notably in occupational settings and from the use of consumer products containing benzene. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods to estimate exposure of identifiable population subgroups from all sources simultaneously have not yet been developed. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various benzene concentrations. The 70-kg man is used for estimating intake.

a. Water

Cumulative estimates of the U.S. populations exposed to various benzene levels in drinking water from public drinking water systems are presented in Table IV-I. The values in the table were obtained using Federal Reporting Data Systems data on populations served by primary water supply systems (FRDS 1983) and the estimated number of these water systems that contain a given

Table IV-1. Total Estimated Cumulative Population (In Thousands) Exposed to Benzene in Drinking Water Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of:											
		≥0.5	>5	>10	>20	>30	>40	>50	>60	>70	>80	>90	>100
Groundwater	73,473	1,037	155	49	6.5	3.2	0.5	0.25	0.25	0.05	0	0	0
Surface water	140,946	3,792	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0	0	0
Total	214,419	4,829	155	49	6.5	3.2	0.5	0.25	0.25	0.05	0	0	0
(% of total)	(100%)	(2.3%)	(<0.1%)	(<0.1%)	(<0.1%)	(<0.1%)	(<0.1%)	(<0.1%)	(<0.1%)	(<0.1%)	(0%)	(0%)	(0%)

level of benzene. An estimated 4,829,000 individuals (2.3% of the population of 214,419,000 using public water supplies) are exposed to levels of benzene in drinking water at or above 0.5 ug/l, while 155,000 individuals (< 0.1%) are exposed to levels above 5 ug/l. It is estimated that 3,200 individuals are exposed to levels greater than 30 ug/l. Of the approximately 4.7 million people exposed to levels ranging from 0.5 to 5 ug/l, 3.8 million (79%) obtain water from surface water supplies. However, all exposure to benzene in drinking water at levels above 5 ug/l is expected to be from groundwater sources.

No data were obtained on regional variations in the concentration of benzene in drinking water. The highest concentrations are expected to occur near sites of oil spills and solvent use and also, in the case of groundwater, near waste disposal sites.

Daily intake levels of benzene from drinking water were estimated using various exposure levels and the assumptions presented in Table IV-II. The data in the table suggest that the majority of the persons using public drinking water supplies would be exposed to intake levels below 0.014 ug/kg/day.

Table IV-II. Estimated Drinking Water Intake of Benzene

Exposure level (ug/l)	Persons using supplies exposed to indicated levels		Intake (ug/kg/day)
	Population	% of Total population	
$\geq$0.5	4,829,000	2.3%	$\geq$0.014
>5.0	155,000	<0.1%	>0.14
>10	49,000	<0.1%	>0.29
>40	500	<<0.1%	>1.1

Assumptions: 70-kg man, 2 liters of water/day.

An indication of the overall exposure of the total population to benzene can be obtained through the calculation of population-concentration values. These values are a summation of the individual levels of benzene to which each member of the population is exposed. An explanation of the derivation of these values is presented in Appendix C. The estimates were 3.5×10^6 ug/l x persons (best case), 1.5×10^7 ug/l x persons (mean best case), 1.2×10^8 ug/l x persons (mean worst case), and 1.3×10^8 ug/l x persons (worst case).

Assuming a consumption rate of 2 liters of water/day, population-exposure values of 7.0×10^6 ug/day x persons (best case), 3.0×10^7 ug/day x persons (mean best case), 2.4×10^8 ug/day x persons (mean worst case), and 2.6×10^8 ug/day x persons (worst case) were derived.

b. Diet

Little information was obtained on the dietary intake of benzene. Of the few foods with quantified levels of benzene, eggs contained the highest amount; one egg may contain as much as 100 ug of benzene (Drill and Thomas 1979).

Dietary benzene intake as high as 250 ug/day has been estimated by the National Cancer Institute from beef, eggs, and rum alone (Drill and Thomas 1979). Assuming that the average adult male weighs 70 kg, an intake of 250 ug/day would be equivalent to 3.6 ug/kg/day. In the absence of further data, the dietary intake of benzene was assumed to be at that level. Variances in individual exposure due to differences in diet could not be assessed.

It was expected that dietary levels of benzene would vary somewhat with geographical region, with higher levels occurring in foods from areas near sources of benzene. However, since benzene may occur naturally in foods (Drill and Thomas 1979), geographical variations may be overshadowed by background levels present in the foods. Because of the limited data, no estimates of variations in intake by geographical region could be made.

c. Air

Exposure to benzene in the atmosphere varies from one location to another. The highest level of benzene reported in the atmosphere was 1,100 ug/m^3 (Pellizzari 1979 cited in Brodzinsky and Singh 1982). High levels, averaging greater than 100 ug/m^3, have been detected in other areas. Normal levels, however, are somewhat lower. Brodzinsky and Singh (1982) calculated median air levels of benzene for rural/remote areas, urban/suburban areas, and source dominated areas of 4.5, 8.9, and 9.6 ug/m^3, respectively.

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The monitoring data available are not sufficient to determine regional variations in exposure levels for benzene. However, urban and industrial areas appear to contain higher levels, as expected.

The daily respiratory intake of benzene from air was estimated using the assumptions presented in Table IV-III and the median and maximum levels for benzene reported above. The estimates in Table IV-III indicate that the daily benzene intake from air for adults in source dominated areas is approximately 3 ug/kg/day. In contrast, the intake calculated using the maximum level reported is 360 ug/kg/day; few if any persons are believed to be exposed at that level. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Table IV-III. Estimated Respiratory Intake of Benzene

Exposure (ug/m^3)	Intake (ug/kg/day)
Rural/remote (4.5)	1.5
Urban/suburban (8.9)	2.9
Source dominated (9.6)	3.2
Maximum (1,100)	360

Assumptions: 70-kg man, 23 m^3 of air inhaled/day (ICRP 1975).

In addition to the available monitoring data, Mara and Lee (1978) have provided estimates of atmospheric levels of benzene and the size of the exposed population by applying air dispersion models to several benzene emission sources. The computed average annual concentrations and the size of the populations exposed from each source are presented in Tables IV-IV and IV-V.

The data in Table IV-IV, which indicate that about half of the U.S. population is exposed to average benzene concentrations between 0.3-13 ug/m^3, were calculated by assuming that the individuals exposed remain at one location (i.e., their residence) 24 hours per day. Table IV-V provides for several scenarios concerning mobility of individuals (i.e., time is spent in several locations rather than 24 hours at their residence). These latter assumptions shift the estimated distributions to suggest that half of the U.S. population is exposed to average concentrations between 3.5-13 ug/m^3.

Table IV-IV. Summary of Estimated Population Exposures to Atmospheric Benzene from Specific Benzene Emission Sources[a]

Source	Population exposed to benzene concentrations (ug/m^3)[b]					Comparison among sources (10^6 ug/m^3-person-years)[b]
	Annual average					
	0.3-3.2	3.5-13	13-32	> 32	Total[c]	
Chemical manufacturing	6,000,000	1,000,000	200,000	80,000	7,300,000	27
Coke ovens	300,000				300,000	0.6
Petroleum refineries	5,000,000	3,000			5,000,000	8.0
Solvent operations	d				--	--
Storage and distribution of gasoline	e				--	--
Automobile emissions - urban	69,000,000	45,000,000			110,000,000	480
Gasoline service stations - urban	30,000,000	2,000,000			32,000,000	61
People using self-service gasoline				f	37,000,000	5.1

[a] Persons living in the vicinity of benzene sources are assumed to spend all their time in that location.

[b] Data converted from ppb to ug/m^3 by multiplying by 3.2.

[c] Population estimates are not additive vertically because of double-counting. Totals are rounded to two significant figures.

[d] Exact determination is impossible.

[e] Annual average estimated at << 0.3 ug/m^3. The population exposed was not determined but is assumed to be very small.

[f] Estimated at 780 ug/m^3 for 1.5 hr/year/person.

Source: Mara and Lee 1978

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Table IV-V. Summary of Estimated Total Exposures[a] of Persons Residing in the Vicinity of Atmospheric Benzene Sources

	Number exposed					Comparison among sources[b,c] (10^6 ug/m^3-person-years)
	Annual average benzene concentration (ug/m^3)					
Vicinity of residence	0.3-3.2	3.5-13	13-32	> 32	Total[b]	
Chemical manufacturing	3,900,000	3,100,000	200,000	80,000	7,300,000	32
Coke ovens	200,000	100,000			300,000	0.6
Petroleum refineries	3,250,000	1,750,000			5,000,000	14
Urban areas		110,000,000			110,000,000	800

[a] The term "total exposure" is the sum of an individual's exposure to atmospheric benzene from a variety of activities during a year. It is assumed that people spend part of their time away from their residence, resulting in exposures to different benzene concentrations depending on their activity (i.e., commuting to work, shopping, traveling to personal business). Nonurban exposures are not included in this analysis, but are expected to range from undetectable to 3.2 ug/m^3.

[b] Rounded to two significant figures.

[c] Median values were used instead of the midpoint of the ranges to allow better comparison with Table IV-IV.

Source: Mara and Lee 1978

The tables also present population-concentration estimates for benzene. The addition of individual population-concentration estimates in Table IV-IV results in a combined value of 5.8 x 10^8 ug/m^3 x persons; the addition of estimates in Table IV-V results in a value of 8.5 x 10^8 ug/m^3 x persons. [It should be noted that this addition may result in some double-counting (Table IV-IV).] Assuming an inhalation rate of 23 m^3 of air/day, a population-exposure of 1.3 x 10^{10} ug/day x persons (Table IV-IV) or 2.0 x 10^{10} ug/day x persons (Table IV-V) was calculated.

SUMMARY

Table IV-VI presents a general view of the total amount of benzene received by an adult male from air, food, and drinking water. Four separate exposure levels in air, five exposure levels in drinking water, and one exposure level from foods are shown in the table.

The data presented have been selected from an infinite number of possible combinations of concentrations for the three sources. The actual exposures encountered would represent some finite subset of this infinite series of combinations. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to benzene at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

Brodzinsky and Singh (1982) calculated a median urban/suburban air level of benzene of 8.9 ug/m^3 based on air monitoring data. Assuming an air level of 8.9 ug/m^3 and the estimated benzene intake of 3.6 ug/kg/day from foods, drinking water would be the predominant source of benzene exposure in the adult male only at drinking water levels above 230 ug/l. An accurate assessment of the number of individuals for which drinking water is the predominant source of exposure cannot be determined from the data since specific locations containing high concentrations of benzene in drinking water and low concentrations of benzene in ambient air and food are unknown.

Population-exposure estimates for benzene in drinking water and air were presented previously. Estimates for drinking water ranged from 0.07-2.6 x 10^8 ug/day x persons; estimates for ambient air ranged from 1.3-2.0 x 10^{10} ug/day x persons. These estimates suggest that ambient air may be a greater source

Table IV-VI. Estimated Intake of Benzene from the Environment by Adult Males in ug/kg/day (% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remote (4.5 ug/m^3)	Urban/suburban (8.9 ug/m^3)	Source dominated (9.6 ug/m^3)	Maximum (1,100 ug/m^3)
0	5.1 (0%)	6.5 (0%)	6.8 (0%)	360 (0%)
0.5[a]	5.1 (0.27%)	6.5 (0.1%)	6.8 (0.1%)	360 (0.004%)
5.0[b]	5.2 (2.7%)	6.6 (2.1%)	6.9 (2.0%)	360 (0.04%)
10[c]	5.4 (5.4%)	6.8 (4.3%)	7.1 (4.1%)	360 (0.08%)
40[d]	6.2 (18%)	7.6 (14%)	7.9 (14%)	360 (0.31%)

Intake from each source (see Sections 5.1-5.3):

Water:	0.5 ug/l:	0.014 ug/kg/day
	5.0 ug/l:	0.14 ug/kg/day
	10 ug/l:	0.29 ug/kg/day
	40 ug/l:	1.1 ug/kg/day
Air:	4.5 ug/m^3:	1.5 ug/kg/day
	8.9 ug/m^3:	2.9 ug/kg/day
	9.6 ug/m^3:	3.2 ug/kg/day
	1,100 ug/m^3:	360 ug/kg/day
Food:		3.6 ug/kg/day

[a] 4,829,000 individuals using public drinking water systems are estimated to be exposed to levels ≥ 0.5 ug/l (2.3% of population using public water supplies).

[b] 155,000 individuals using public drinking water systems are estimated to be exposed to levels > 5.0 ug/l (< 0.1% of population using public water supplies).

[c] 49,000 individuals using public drinking water systems are estimated to be exposed to levels > 10 ug/l (< 0.1% of population using public water supplies).

[d] 500 individuals using public drinking water systems are estimated to be exposed to levels > 40 ug/l (<< 0.1% of population using public water supplies).

of exposure to benzene than drinking water on a general population basis. Comparison of these estimates, however, may be deceiving since the same population-exposure level can occur if: 1) a whole population is exposed to moderate levels of a chemical or 2) some segments of the same population are exposed to high levels and others to low levels. The population-exposure values presented give no indication of the relative predominance of drinking water and air as specific sources of benzene on a site-by-site or subpopulation basis.

The relative source contribution data are based on estimated intake and do not account for a possible differential absorption rate for benzene by route of exposure. The relative dose received may vary from the relative intake. In addition, the relative effects of the chemical on the body may vary by different routes of exposure.

REFERENCES

Brodzinsky R, Singh HB. 1982. Volatile organic chemicals in the atmosphere: An assessment of available data. Prepared by SRI International, Menlo Park, CA, for Environmental Sciences Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC. Contract No. 68-02-3452.

Drill S, Thomas R. 1979. Environmental sources of benzene exposure: Source contribution factors. Prepared by Mitre Corporation for the U.S. Environmental Protection Agency. EPA-570/9-79-004.

FRDS. 1983. Federal Reporting Data System. Facilities and population served by primary water supply source (FRDS07), April 19, 1983. U.S. Environmental Protection Agency, Washington, DC.

ICRP. 1975. International Commission on Radiological Protection. Report of the task force of reference man. ICRP Publication 23. New York: Pergamon Press.

Letkiewicz F, Johnston P, Macaluso C, Elder R, Yu W, Bason C. 1983. Occurrence of benzene in drinking water, food, and air. Prepared by JRB Associates, McLean, VA, for Office of Drinking Water, U.S. Environmental Protection Agency, Washington, DC. EPA Contract No. 68-01-6388.

Mara SJ, Lee SS. 1978. Assessment of human exposures to atmospheric benzene. Prepared by SRI International for Office of Air Quality Planning and Standards, U.S. Environmental Protection Agency, Research Triangle Park, NC. EPA-450/3-78-031.

Pellizzari ED. 1979. Information on the characteristics of ambient organic vapors in areas of high chemical production. Prepared by Research Triangle Institute, Research Triangle Park, NC, for U.S. Environmental Protection Agency. Cited in Brodzinsky and Singh 1982.

IV. PHARMACOKINETICS/METABOLISM

A. Excretion of Unchanged Benzene

Benzene toxicity in humans is usually caused by inhalation of ambient air containing benzene vapor. Following cessation of exposure the body burden of benzene is reduced either by exhaling benzene in the expired air or by metabolism. The exhalation of unchanged benzene has been studied in dogs (Schrenk, et al., 1941), rabbits (Parke and Williams, 1953), mice (Andrews, et al., 1977a) and rats (Rickert, et al., 1979). Schrenk, et al. (1941) exposed dogs to 800 ppm benzene by inhalation and determined that the time was related to the duration of exposure because of the tendency of benzene to accumulate in body fat. Parke and Williams (1953) administered ^{14}C-benzene orally and recovered approximately 43 percent of the administered dose as unmetabolized benzene in trapped exhaled air. Rickert, et al. (1979) reported that the excretion of unchanged benzene from the lungs of rats followed a biphasic pattern suggesting a two-compartment model for distribution and a $t_{1/2}$ of 0.7 hr. This agreed with experimental $t_{1/2}$ values for various tissues which ranged from 0.4 to 1.6 hr. Andrews, et al. (1977a) administered benzene to mice subcutaneously and recovered 72 percent of the dose in the air. Simultaneous treatment with both benzene and toluene (Andrews, et al., 1977a; Sato and Nakajima, 1979b) or benzene and piperonyl butoxide (Timbrell and Mitchell, 1977) increases the excretion of unchanged benzene in the breath.

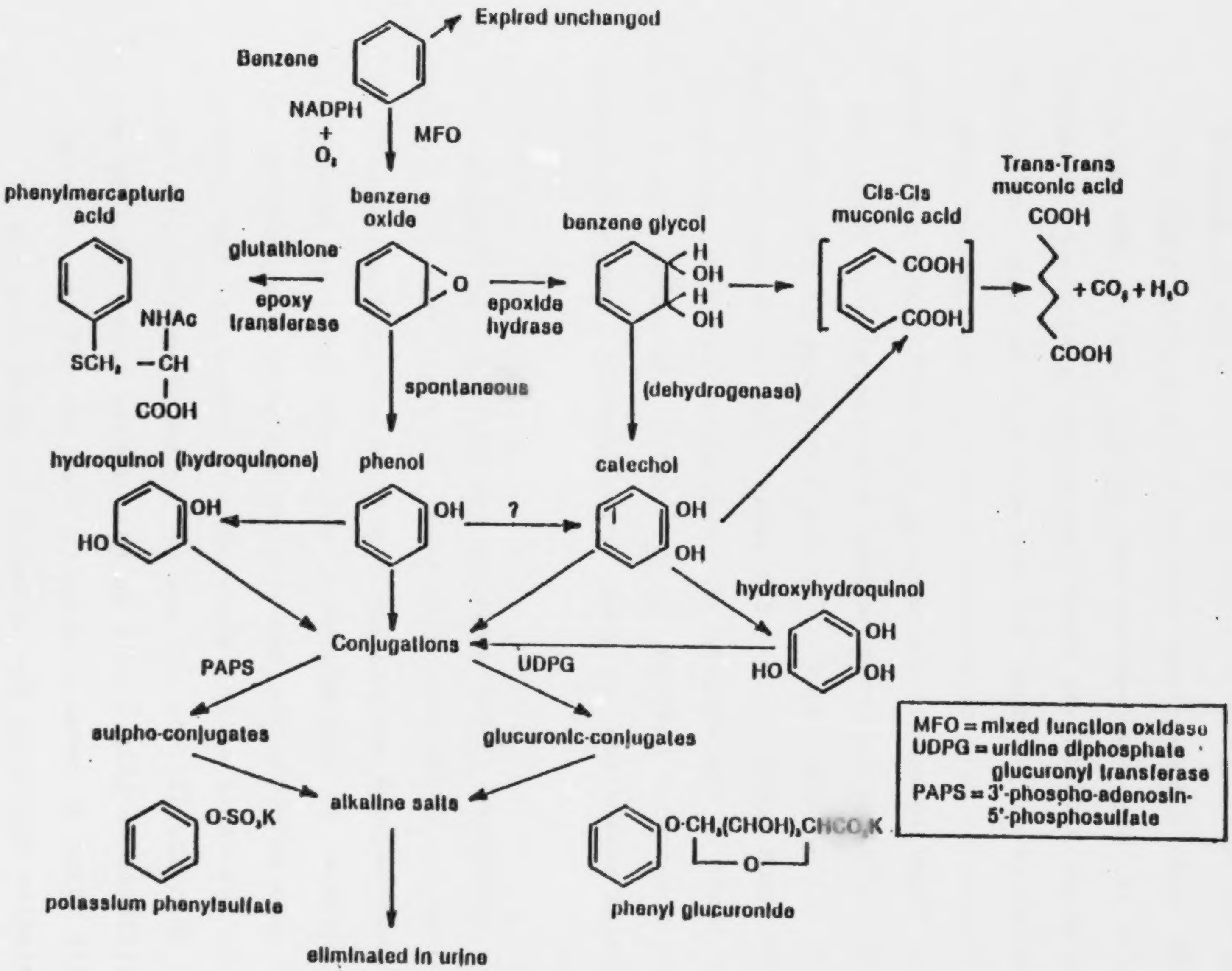
These compounds appear to act by inhibition of benzene metabolism which thereby leaves more benzene available for excretion through the lungs.

B. Metabolism of Benzene

The metabolic pathway for benzene, as it is currently understood, is shown in Figure 1. Unlike some previous reports of this pathway no figures are given indicating the percentage of each of these metabolites which are formed because there is great variability in these estimates. The primary causes of the variability are the dose dependency of excretion of unchanged benzene versus its conversion to metabolites and the species studied.

It has been shown since the latter part of the nineteenth century that benzene is biologically converted to phenol (Schultzen and Naunyn, 1867) as well as to catechol and hydroquinone (Nencki and Giocosa, 1880). The first detailed studies of the metabolites of benzene formed *in vivo* were reported by Porteous and Williams (1949a,b), and with the advent of ^{14}C-benzene these studies were improved upon by Parke and Williams (1953). Extensions of this work in recent years have largely concentrated on metabolism in various animal species, on the mechanism of benzene metabolism using *in vitro* techniques and on attempting to relate benzene metabolism to its toxicity (Snyder and Kocsis, 1975; Snyder, *et al.*, 1977).

In a landmark series of papers (Porteous and Williams, 1949a,b; Parke and Williams, 1953) R.T. Williams outlined the


Benzene
Expired unchanged
NADPH + O₂
MFO
benzene oxide
phenylmercapturic acid
glutathione
epoxy transferase
SCH₂ —CH NHAc COOH
epoxide hydrase
benzene glycol
Cis-Cis muconic acid
COOH
COOH
Trans-Trans muconic acid
COOH
COOH
+ CO₂ + H₂O
spontaneous
(dehydrogenase)
hydroquinol (hydroquinone)
phenol
catechol
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Conjugations
PAPS
UDPG
sulpho-conjugates
glucuronic-conjugates
alkaline salts
O-SO₃K
potassium phenylsulfate
O-CH₂(CHOH)₃CHCO₂K
O
phenyl glucuronide
eliminated in urine
MFO = mixed function oxidase
UDPG = uridine diphosphate glucuronyl transferase
PAPS = 3'-phospho-adenosin-5'-phosphosulfate

FIGURE 1 METABOLIC PATHWAY OF BENZENE IN LIVER

/boader aspects of benzene metabolism in rabbits by identifying most of the metabolites in urine as well as those in expired air. He later demonstrated that about one percent could be recovered in bile (Abou-el-Marakem, et al., 1967). The major hydroxylation product was phenol which, along with some catechol and hydroquinone, is found for the most part in urine conjugated with ethereal sulfate or glucuronic acid. Unconjugated phenol has been found in mouse (Andrews, et al., 1977a) and rat (Cornish and Ryan, 1968) urine after benzene administration. Parke and Williams (1953) also reported on the occurrence of phenylmercapturic acid and muconic aid. The later, along with labeled carbon dioxide found in the expired air, suggested that some opening of the ring occurred. Andrews, et al. (1977a) estimated that a 25 g mouse could metabolize, at most, approximately 1 mmole of benzene per day.

Longacre (1980) compared the excretion of benzene metabolites in C57/B6 and DBA/2 mice. He found that the patterns of metabolites were qualitatively similar when the urine was chromatographed on DEAE-sephadex, but closer analysis demonstrated that although the two strains excreted equal amounts of catechol, the DBA/2 animal excreted more phenol and less hydroquinone than the C57/B6. It has yet to be determined whether these differences play a role in the greater susceptibility of the DBA/2 mouse to benzene toxicity than the C57/B6. However, these studies emphasize the necessity to reevaluate the

the metabolites of benzene for each new species or strain of animals in which benzene toxicity is studied.

Benzene metabolism has been studied in liver homogenates (Snyder et. al., 1967; Hirokowa and Nomiyama, 1962; Sakamoto et. al., 1957), cell supernatant fractions containing microsomes (Snyder et.al., 1967; Kocis et.al., 1968; Sakamoto et.al., 1957; Sato and Nakajima, 197a,b) and microsomes (Posner et.al., 1961; Snyder et.al.,1967; Gonasun et. al.,1973; Drew et.al., 1974; Harper et.al., 1975; Tunek et.al., 1978). It is clear from these studies that benzene is metabolized in liver microsomes of rat, rabbit, and mouse. Gonasun et.al. (1973) demonstrated the first step is mediated by the mixed function oxidases. Jerina and coworkers (Jerina et. al., 1968; Jerina and Daly, 1974) have outlined a pathway for benzene metabolism which revolves about the formation of benzene oxide, an epoxide of benzene, as the first product (Figure 1). This highly unstable intermediate rearranges non-enzymatically to form phenol as the major metabolite of benzene found in urine. Catechol formation is thought to result from the hydration of benzene oxide by the enzyme epoxide hydratase followed by oxidation to catechol. The intermediate dihydrodiol was observed in rat urine by Sato et. al. (1963). The enzyme dihyrdodiol dehydrogenase has been identified and purified by Vogel et. al. (in press) and is thought to mediate the oxidation of benzene of benzene dihydrodiol to catechol. The evidence for the epoxide intermediate is that the addition of the epoxide to liver preparations yields the same metabolites as benzene (Jerina et. al.,

1968) and the addition of excess hydratase enzyme increases the formation of catechol (Tunek, *et al.*, 1978). Thus, it appears that different metabolic pathways.

The metabolic pathway leading to the formation of hydroquinone has yet to be established. It may be formed via passage of phenol through the mixed function oxidase but other enzymatic steps have not been ruled out. There is greater likelihood, on the basis of *in vitro* studies that the premercapturic acid, i.e., the glutathione conjugate, is formed by the addition of glutathione to the epoxide and the reaction is mediated by the glutathione transferase enzyme (Jerina, *et al.*, 1968).

The metabolism of benzene in liver preparations, i.e., homogenates, 9,000 g supernatants or microsomes, can be stimulated by treating animals with enzyme inducing agents prior to sacrifice. Benzene (Snyder, *et al.*, 1967; Saito, *et al.*, 1973; Gonasum, *et al.*, 1973), phenobarbital (Snyder, *et al.*, 1967; Ikeda and Ohtsuji, 1971; Drew and Fouts, 1974; Gut, 1978; Tunek, *et al.*, 1979; Tunek and Oesch, 1979), 3-methylcholanthrene (Drew and Fouts, 1974), DMSO (Kocsis, *et al.*, 1968) and chlordiazepam, diazepam and oxazepam (Jablonska, *et al.*, 1975) all induce benzene hydroxylase activity. The *in vivo* significance of inducing benzene metabolites has been questioned by Gut (1978) who has argued that induction of microsomal benzene metabolism may not be reflective of the overall raste of benzene metabolism because *in vitro* systems do not provide an accurate picture of the pharmacokinetics observed *in vivo*. Further studies in which the effects of these inducers on metabolism *in vivo* and *in vitro* in the same experiment as well as on toxicity of benzene will be required to clarify this issue.

Conversely, direct addition of any of several chemicals to the liver preparations *in vitro* can inhibit benzene metabolism. Thus, carbon monoxide, aniline, metyrapone, SKF525A, aminopyrine, cytochrome *c* (Gonasun, 1973), aminotriazole (Hirokawa and Nomiyama, 1962) and toluene (Andrews, *et al*., 1977a) have been shown to interfere with benzene metabolism *in vitro*.

The unique behavior of the mixed-function oxidase system that metabolizes benzene has recently been described (Tunek and Oesch, 1979). These experiments, which employed control, benzene-induced and phenobarbital-induced rat liver microsomes, determined the effects of either metyrapone or Renex 190 *in vitro* on 7-ethoxycoumarin deethylation and benzene hydroxylation. As expected, it was found that metyrapone or Renex 190 treatment inhibited 7-ethoxycoumarin metabolism in control microsomes. Considerably more inhibition was observed in phenobarbital-induced microsomes. Pretreatment with benzene, however, resulted in no greater inhibition than with control microsomes. Furthermore, addition of either metyrapone metyrapone or Renex 190 to microsomes increased benzene hydroxylation in both control and benzene-induced microsomes but not in phenobarbital-induced microsomes. It is significant that while Tunke and Oesch found that metyrapone increased benzene metabolism in rat liver microsomes, Gonasun, *et al*. (1973) found that metyrapone inhibited benzene metabolism in mouse liver microsomes. While phenobarbital pretreatment may induce at least one of these monooxygenases, benzene pretreatment may induce the benzene monooxygenase forms(s) that is (are) normally present in control microsomes.

Miller and Miller (1977) have for many years propounded the concept that the toxicity or carcinogenicity of many xenobiotics may result from the metabolic conversion of the xenobiotic to a chemically reactive intermediate which covalently binds to either cellular protein or nucleic acids. Adverse effects ensue as a result of these structural alterations unless they can be repaired by the various mechanisms available to the cell. Snyder, et al. (1978) have studied the time course of the irreversible binding of ^{3}H-benzene metabolite(sl) to proteins in both mouse liver and bone barrow. Covalent binding of benzene-dervied radioactivity increased both with dose and frequency of dosing. Eventually the binding in liver reached a plateau of the binding in bone marrow decreased as benzene toxicity became severe. The decrease in protein binding in the marrow was explained by a replacement of protein by fat as the marrow became hypoplastic. Other laboratories have also studied tissue binding of radioactivity after labeled benzene administration. Irons, et al. (1980) observed both benzene matbolism and covalent binding to cellular marcomolecules using the *in situ* bone marrow preparation cited previously. Lutz and Schlatter (1977) have shown that inhaled radiolabeled benzene can covalently bind to rat liver DNA. Benzene was bound to the extent of 2.38 umoles/mole DNA phosphate. Tunek, et al. (1979) have separated microsomal proteins after incubation with labeled benzene and have partially characterized the proteins to which benzene binds.

Tunek, et al. (1978) found that the addition of either glutathione or cysteine to microsomal preparations which metabolize benzene had little effect on phenol formation but inhibited covalent binding to microsomal protein. They suggested that the immediate precusor to covalent binding was not benzene oxide but a metabolite of phenol. In a subsequent effort (Tunek, et al., in press) have shown that the quinone and semi-quinone derivatives of hydroquinone readily form glutathione conjugates and have postulated that these may be closer approximations of the actual reactive metabolites. A similar mechanism was recently suggested by Irons, et al. (in press).

C. Disposition of Benzene in Humans

The most frequent role of exposure to benzene by humans is via inhalation. Toxic effects in humans have often been attributed to combined exposure by both respiration and through the skin. Thus, rotogravure workers were described as washing ink from their hands in open vats of benzene (Hunter, 1962). Although Lazarew, et al. (1931) claimed that benzene could be absorbed by rabbits through the skin neither Cesaro (1946) nor Conca and Maltagliati (1955) could demonstrate significant cutaneous absorption in humans. Nevertheless, small amounts of benzene absorbed by this route may not have been detected.

Following exposure to benzene, humans, like animals, eliminated unchanged benzene in the expired air (Sherwood and Carter, 1970; Hunter, 1968; Nomiyama and Nomiyama, 1974a, 1974b; Sato and Nakajima, 1979b; Srbova, et al., 1950). The elimination of unchanged benzene was quantitated in a series of studies by Nomiyama and Nomiyama (1974a, 1974b) who exposed men and women to benzene at levels of 52-62 ppm for four hours and determined its respiratory disposition. A mean value of 46.9% of the benzene was taken up in these subjects, 30.2% was retained and the remaining 16.8% was excreted an unchanged benzene in the expired air. Pharmacokinetic plots of respiratory elimination were interpreted to indicate that there were three phases to the excretion described by three rate constants. There were no significant differences between men and women in these studies. Hunter (1968) who exposed humans to benzene at 100 ppm detected benzene in expired air 24 hours later and suggested that it was possible to back extrapolate to the concentration of benzene in the inspired air.

Determination of benzene metabolism in humans was first evaluated as a measure of exposure. Yant, et al. (1936) suggested that since benzene metabolites in the urine could be detected as ethereal sulfates it would be possible to estimate benzene exposure by measuring the ration of inorganic to organic sulfate. Normally the inorganic sulfate is present at about four times the organic levels. Exposure tends to increase the organic sulfate and lower the inorganic. Hammond and Herman (1960) suggested that of total sulfates, inorganic sulfates of 80-95% were normal, 70-80% indicated some exposure to benzene, 60-70% suggested a dangerous level of benzene exposure and 0-60% indicated that benzene levels were sufficiently high to provide an extremely dangerous atmosphere for humans.

In humans the sulfate is the major conjugate of phenol until levels of approximately 400 mg/L are reached (Sherwood, 1972). Beyond that level glucuronides are seen. Teisinger, et al. (1952) exposed humans to benzene at 100 ppm for 5 hours and found that the urine contained primarily phenol with small amounts of catechol and hydroquinone. It would appear that benzene metabolism in humas is similar to that in animals with respect to the production of the major metabolites phenol, catechol and hydroquinone.

V. RELATIVE SOURCE CONTRIBUTION

Incorporated in Chapter III - Human Exposure

VI. HEALTH EFFECTS IN ANIMALS

A. Acute and Chronic Effects

The early reports of Santesson (1897) and Selling (1916) of benzene toxicity in humans were accompanied by descriptions of experimentally induced benzene toxicity in animals. The observations of pancytopenia and bone marrow depression in animals, similar to those in man following chronic exposure to benzene, suggested that animal models would be useful in the study of the mechanism of benzene toxicity.

Santesson (1897) and Selling (1916) were able to reproduce benzene toxicity in animals best when they administered the benzene subcutaneously.

The first demonstration of benzene toxicity in animals that were administered benzene by inhalation was reported by Weiskotten and his colleagues (1920) who exposed rabbits and demonstrated findings essentially similar to those reported by Santesson and Selling. Although Weiskotten did not report the air concentration of benzene in his studies, his description of the apparatus and of the amount of benzene used in his experiments permit us to calculate the dose of benzene by applying a first order differential equation (Snyder and Kocis, 1975). It appears that he was able to demonstrate benzene toxicity by exposing rabbits to a mean benzene concentration of 240 ppm. In later experiments it was shown that dogs exposed to 600 to 1000 ppm (Hough and Freeman, 1944) developed leukopenia mice developed fatal anemia and leukopenia within 12-15 days at similar air levels (Petrini, 1941) and rats, guinea pigs, rabbits and monkeys exposed to 80-85 ppm developed leukopenia (Wolf, et al., 1956). Leukopenia was also reported in similar studies when rats or dogs were exposed to benzene at 1000 ppm (Nau, et al., 1966; Svirbely, et al., 1944). Deichmann, et al. (1963) showed that when exposing rats to benzene vapor at 831, 65 and 61 ppm significant leukopenia was observed within 2 to 4 weeks; at 47 and 44 ppm a less severe leukopenia was observed at 5-8 weeks; no leukopenia was observed when the animals were exposed to 31 ppm for 4 months, 29 ppm for 3 months, or 15 ppm for 7 months. It has been argued that the duration of these studies may not have been long enough to demonstrate leukopenia at the lower doses but there does indeed appear to be a dose times time relationship for the production of toxicity. Ikeda (1964) and Drew, et al. (1974) reported that repetitive dosing of rats with 1000 ppm and 1650 ppm, respectively, produced leukopenia. The data suggest that barring cases of hypersensitivity there is in all likelihood a relationship between dose, time of exposure and degree of toxicity in various species (Steinberg, 1949). Four reports have been chosen to exemplify this relationship. Latta and Davies (1941) administered benzene to rats subcutaneously at doses of 1760-3520 mg (2-4 ml) kg/day whereas Deichmann, et al. (1963) exposed rats to benzene in the atmosphere and observed toxic effects in the range of 65-831 ppm. Selling (1916) gave rabbits benzene subcutaneously at a dose of 880 mg (1 ml) kg while Weiskotten, et al. (1920) exposed rabbits to atmospheric benzene at a level which has been calculated to be 240 ppm (Snyder and Kocsis, 1975). Although an initial transitory leukocytosis was frequently observed the eventual result in each case regardless of the species or the route of administration was leukopenia. At lower doses more time was required but the eventual result was the same. The predominant effect was neutropenia accompanied by an apparent lymphocytosis which gradually disappeared as benzene attacked lymphoid tissue. Latta and Davies (1941) reported that lymphatic tissue was more sensitive to benzene than myeloid tissue in rats while Selling (1916) reported that the opposite was true in rabbits. The neutropenia is characterized by a shift to the left in the Arneth count which suggests that leukocyte maturation is impaired. The leukopenia can occur rapidly and cell counts may reach extremely low levels prior to death.

It is significant that the recent extensive studies of Snyder, et al. (1978, 1980) in large measure confirmed these earlier studies. In these studies Sprague-Dawley rats and both AKR/J and C57BL/6J mice were exposed to benzene by inhalation at concentrations of either 100 ppm or 300 ppm 6 hours per day, five days per week for life. In the first report they showed that the rats exhibited lymphocytopenia, mild and decreased survival time. The mice also demonstrated lymphocytopenia, anemia and decreased survivial but these were accompanied by granulocytosis and reticulocytosis. The second report, in addition to commenting upon evidence for benzene induced carcinogenicity, also showed that benzene induced bone marrow hypoplasia, anemia and lymphocytopenia.

There is only one study of benzene induced hematotoxicity where the benzene was given orally. Wolf, et al. (1956) used matched groups of 10 female rats and administered either olive oil or benzene emulsified in 5% gum arabic. Thus, the benzene was in effect given in water. In all, 132 doses were given over a 187 day treatment period as single daily doses. The doses ranged from 1-100 mg/kg/day. No effects were seen at 1 mg/kg, slight leucopenia at 10 mg/kg, and both leucopenia and anemia at 50 and 100 mg/kg. No cell counts were reported. It may be concluded that in these studies the threshold for benzene toxicity was between 1 and 10 mg/kg. It is clear that further study of the effects of benzene given in water is required to confirm the studies of Wolf, et al. (1956), to relate the effects of benzene given by other routes with those produced by benzene given orally and to use the data in attempting to extrapolate to man.

The use of animal models to determine which cell type is most sensitive to benzene in order that monitoring of that cell type in man might be used to monitor benzene toxicity may in retrospect have been relatively unprofitable. In man, arguments have been advanced to demonstrate that each of the cell types may be an early indicator of benzene exposure if their levels in circulating blood decrease. In animals, larger doses of benzene have been given than those to which man is exposed in an attempt to condense the course of the disease in time. Because of the extremely long half life of the red cell it is difficult to observe changes in red cell counts during benzene exposure unless animals were exposed to low doses for long period of time (e.g., Snyder, et al., 1978, 1980). Platelets are rather more difficult to measure and are not commonly used as indicators of benzene exposure. Therefore, leukocytes √ which suffer from neither drawbacks, have usually been reported in animal studies as the first cell type to be depleted. It must be stressed, however, that no conclusive evidence exists to show that benzene preferentially depresses the production of any individual cell line in the bone marrow.

Attempts to study which circulating cell precursors were most sensitive to benzene required an understanding of the function of the bone marrow. For each of the three major cell types, the processes of cell maturation and proliferation must function if an adequate number of each cell type is to reach the circulation as a mature, functional cell. The fundamental cell of the marrow, called the pluripotential stem cell, gives rise to each of the cell lines and the process is controlled by a complex series of interactions including hormonal influences, the microenvironment of the bone, feedback mechanisms and probably other forces as well. Once a stem cell is committed to the eventual formation of a specific type of mature cell, it commences to mature and to undergo mitosis to insure that the process of amplification results in an appropriate number of mature cells. In the theory it is possible to interfere with cell development by preventing these functions at any stage of cell maturation.

Attempts to detect the stage of cell maturation most sensitive to benzene have led to similar conclusions. Steinberg (1949), Moeschlein and Speck (1967), Rondanelli, et al. (1970) and Lee, et al. (1973) agree that the cells most sensitive to benzene are the early committed cells actively engaged in cell proliferation and maturation. Thus, in the red cell line Lee, et al. (1974) suggested that the pronormoblasts and to some extent the normoblasts were the most sensitive whereas undifferentiated stem cells, reticulocytes and mature red cells were relatively resistant to benzene.

Wildman, et al. (1976) however suggested that the reticulocyte may be a target for benezene since the addition to benzene at a concentration of 0.113 M to reticulocytes in vitro resulted in a decrease in heme and protein synthesis. Kahn and Muzyka (1973) reported on changes in porphyrin metabolism in benzene exposed workers after examining delta-aminolevulinic acid (ALA), porphobilinogen (PGB), coproporphyrin (CP) and protoporphyrin (PP) in brain, red cells and plasma of benzene exposed rabbits. ALA, PBG, and PP accummulated in gray matter of brain during chronic treatment with benzene (88 mg/kg, subcutaneously, four times per week for 5-6 months). No changes in red cells or plasma were seen. In 16 of 27 exposed workers an increase in red cell ALA was seen. These studies taken together suggest that more research is needed to determine whether or not benzene can interfere with heme or hemoglobin synthesis at the reticulocyte level, and if humans are sensitive at lower concentrations over time.

The use of colony forming unit (CFU) assays concentrating on bone marrow cells in culture has contributed to our understanding of targets for benzene and some of its hydroxylated derivatives. Several years ago Uyeki, et al. (1977) showed that bone marrow cells taken from BDF_1 mice exposed to benzene by inhalation (4680 ppm, 8 hr.) displayed depleted of CFU-C (leucocyte precursors) on the day following exposure but recovery was evident by seven days. Multiple exposures enhanced the effect. Decreases were also observed using the CFU-S assay (spleen colony forming units, erythroid precursors). Green, et al. (1981) showed that at 103 ppm and higher CD1 mice exposed for 6 hr/day for 5 days displayed a reduction in marrow and spleen cellularity as well as a decrease in GM-CFU-C (granulocytic macrophage colony forming units, committed macrophage precursors) from spleen but not from marrow. At 9.6 ppm for 50 days no changes in marrow activity were seen but splenic cellularity and CFU-S were elevated. At 302 ppm for 26 weeks marrow and spleen cellularity, CFU-S and marrow GM-CFU-C were decreased. Harigaya, et al. (1981) also demonstrated a depression of CFU-S in C57bl/dj mice exposed to benzene (400 ppm, 6 hr/day) for either 9 days intermittently or 11 consecutive days. Wierda, et al. (1981), also using cell culture techniques, have shown that single intraperitoneal injections of benzene resulted in a dose related inhibition of splenic T- and B-lymphocyte responsiveness to mitogenesis. These studies, taken together with those cited above, although apparently relating benzene effects to different primitive cells all indicate that benzene is affecting cell replication and maturation.

B. Immunological Aspects of Toxicity

Early in this century it was recognzied that benzene had an adverse effect on immunological mechanisms. It was demonstrated that susceptibility to tuberculosis (White and Gammon, 1914) and pneumonia (Winternitz and Hirschfelder, 1913; Hirschfelder and Winternitz, 1913) were increased in benzene treated rabbits. The reports of decreased production of red cell lysins, agglutinins for killed typhoid bacilli and opsonins (Simonds and Jones, 1915) and the absence of anti-bacterial antibodies (Camp and Baumgartner, 1915; Hektoen, 1916) in benzene intoxicated rabbits were all indications of depression of the production of various components of the immune mechanism. Developments in the field of immunology have led to studies of the effects of benzene in humans on several immunological components which have been identified in recent years. Smolik and coworkers (Smolik, et al., 1973; Lange, et al., 1973a) studied a large number of workers exposed to but not seriously intoxicated by benzene. They found that serum complement levels, IgG and IgA were decreased but IgM levels did not drop and were in fact slightly higher. These observations taken together with the well-known ability of benzene to depress leukocytes which themselves play a significant role in protection against infectious agents may explain why benzene intoxicated individuals readily succumb to infection and the terminal event in severe benzene toxicity is often an acute overwhelming infection.

These authors also evaluated levels of leukocyte agglutinins and found them elevated in selected individuals exposed to benzene (Lange, et al., 1973b). They extended this observation to suggest that in some persons the picture of benzene toxicity may in part be accounted for as an allergic blood dyscrasia.

Alterations of immunological function may also play a role in the development of acute leukemia resulting from benzene intoxication described above. Current concepts of immunology suggest that a mechanism referred to as "immune surveillance" (Smith, 1970, 1972) is constantly at work to weed out cells which result from mistakes in cellular genetics or genetic changes caused by carcinogenic agents. The mechanism, while not completely understood, appears to involve a recognition of surface components of abnormal cells followed by immunological destruction of the cell or clone or cells. Since some forms of benzene intoxication result in hyperplasis of bone marrow with the occurrence of many bizarre cellular species it may be presumed that some of these may be neoplastic. Damage to immunological mechanisms in benzene toxicity may then impair the immune surveillance response with the resulting development of leukemia.

C. Potential for Mutagenesis and Leukemogenesis

Benzene was found to have no mutagenic potential when tested in Drosophila melanogaster (Nylander, et al., 1978). In this experiment newly hatched larvae were exposed to media containing benzene at a concentration of 1% or 2%. The test system used a stable X-chromosome ($zDpw^{+}le19$) as a control and a genetically unstable sex linked genotype, $sczw^{+}$. Mutation in the two systems is measured by a shift in eye pigmentation; however, neither concentration of benzene showed mutagenic activity.

In a review article, Dean (1978) reported that benzene was tested for mutagenic activity at a concentration of 20 and 600 uL/plate with five Salmonella typhimurium tester strains (TA100, TA98 TA1535, TA1537 and TA1538) with and without metabolic activation by 9,000 g supernatant fractions from liver microsomes. No mutations were observed. Furthermore, higher levels of benzene (0.088-880 mg/plate) also showed no mutagenic effect when S. typhimurium tester strains TA98 and TA1000 were used with the addition of 9,000 g supernatant fractions from both phenobarbital and 3-methylcholanthrene treated rats. Preincubation of benzene with the metabolic activating system also produced negative results. In addition, in this same set of experiments, a host-mediated assay was performed in which mice were pretreated with phenobarbital and given two 0.1 mL subcutaneous injections of benzene. The test organism was S. typhimurium strain TA1950. Again, no increase in mutation rate was observed. In a similar study performed at Litton Bionetics (1977) mutagenic activity of benzene was evaluated using the S. typhimurium and the Saccharomyces cerevisiae tests. No mutations were observed with or without metabolic activation. Benzene also gave negative results in the mouse lymphoma tests but was positive for sister chromatid exchanges in the rat bone marrow assay. It is possible that the mutagenicity of benzene cannot be assessed with bacterial systems because benzene may damage cells. Toluene is routinely used to render the membranes of *Eschirichia coli* permeable to nucleotides while allowing the cells to maintain both their structure and their ability to synthesize DNA (Moses, 1974); however, these bacteria are not viable and changes in the membrane are difficult to detect by bright-field microscopy.

A recent report by Pulkrabek, *et al*. (1980) showed that benzene oxide, the presumed initial metabolite of benzene is mutagenic for *S*. *typhimurium*. While the ability of benzene to cause point mutations is in doubt, production of chromosomal aberrations by benzene have been known for many years (Kissling and Speck; 1969; Tough, *et al*., 1970). Pollini and Colombi (1964a; 1964b) noted a high rate of aneuploid cell production in cultured cells and peripheral lymphocytes from workers displaying severe benzene hemopathy. A number of structural chromosome aberrations were noted in lymphocytes cultured from workers exposed to benzene. Forni, *et al*. (1971a; 1971b) have shown an increased incidence of stable and unstable chromosome aberrations in rotogravure workers exposed to benzene when compared with matched controls. During follow-up studies of these workers the aberrations which persisted were found later to be associated with leukemia. Kahn and Kahn (1973), Funes-Cravioto, *et al*. (1977) and Picciano (1979) investigated cytogenetic effects of industrial exposure to benzene and found increased numbers of chromosomal breaks and figures including rings, dicentrics, translocations and exchange figures in peripheral blood cells. Chromosome aberrations area also common in individuals with benzene associated leukemias (Forni and Moreo, 1967; 1969; Hartwich, et al., 1969; Sellyei and Keleman, 1971).

Tice, et al. (1980) recently demonstrated excessive sister chromatid exchanges (SCE) in DBA/2 mice exposed to 3100 ppm benzene by inhalation for four hours. The SCE frequency, which was approximately doubled, occurred at a level of exposure which did not cause chromosomal aberrations. By comparing the number of SCE found in successive generations of cultured marrow cells, it was determined that SCE occurred both as a result of exposure of previously unexposed cells to benzene and because of the persistence of lesions. The latter is a critical observation for the leukemogenic action of benzene because persistence of such lesions correlates with carcinogenesis in other systems (Goth and Rajewsky, 1974; Nicoll, et al., 1975).

In a recently reported study Meyne and Legator (1980) administered benzen to CD-1 mice either intraperitoneally or via oral gavage at doses of 88, 440, or 880 mg/kg for three days and subsequently measured chromosome aberrations and micronucleus changes. Male mice were more susceptible to chromosome aberrations than females regardless of the route of administrtion. Males were also more sensitive to micronucleii formation when benzene was given orally but not intraperitoneally. Females demonstrated an increase in micronuclei when the benzene was given orally but were less sensitive than the males. They demonstrated no increase when the benzene was given intraperitoneally.

The significance of these studies resides in the finding that benzene has been shown to induce leukemia in humans whereas there is as yet no readily reproducible model for benzene-induced leukemia in animals. Leukemia is known to spontaneously occur with rather high frequency in some strains of mice and there is abundant evidence that chemicals can induce leukemias in both mice and rats (Shay, et al., 1951; Hartman, et al., 1959; Huggins and Sugiyama, 1966; Diwan and Meier, 1976; Ogui, et al., 1976). The reports of Lignaç (1932) in the early 1930's that he had produced leukemia in mice were based on a study in which he treated mice with benzene subcutaneously at a dose of 30 mg/kg for a period of 17-21 weeks. Forty-four mice survived of which 8 were described as having developed leukemia or a lymphosarcoma. Unfortunately experimental details such as strain of mouse and diagonistic criteria are unavailable. Furthermore, no controls were used to correct for spontaneous neoplasms in these animals.

In an attempt to duplicate the results of Lignac, Amiel (1960) treated Akr, DBA_2,C_3H and C57Bl mice with a weekly injection of benzene (30 mg/kg) throughout their entire lifetimes. Neither leukemia nor aplastic anemia were observed. In a more recent study Ward, et al. (1975) used C57Bl mice and after gradually increasing the dose from 450 mg/kg to 1.8 gm/kg continued to treat the animals for 44 weeks, twice weekly with 1.8 gm/kg. For the next 10 weeks a single dose at this level was given. The experiment was terminated 104 weeks after the first injection. Although a number of deaths due to bone marrow depression were observed in the benzene treated animals no significant increase in neoplastic disease was observed when comparing the benzene treated animals with appropriate controls.

Oral dosing of Wistar rats with benzene at doses ranging from 1-100 mg/kg in 132 feedings over a 187 day period resulted in a dose dependent bone marrow depression (Petrini, 1941), but no leukemia. Kirschbaum and Strong (1942) and Laerum (1973) painted benzene on the skin of F strain and hairless mice, respectively, but neither group displayed leukemias during extended observation periods.

Using the inhalation route Wolf, et al. (1956) treated rats (9400 ppm, 1-10 exposures, 1-19 days; 6600 ppm, 70 exposures, 93 days; 4400 ppm, 28 exposures, 38 days; 2200 ppm, 133 exposures, 212 days; 88 ppm, 136 exposures, 204 days), guinea pigs (88 ppm, 193 exposures, 269 days; 88 ppm, 23 exposures, 204 days) and rabbits (80 ppm, 175 exposures, 243 days) using seven hour exposure periods. No instances of leukemia were observed. Jenkins, et al. (1970) exposed 15 rats, 15 guinea pigs and 2 dogs to either 2614 ppm in 30 repeated exposures, 8 hrs/day, 5 days/week; 315 ppm continuously for 90 days; or 179 ppm continuously for 127 days. Again no leukemia was observed.

Two recent reports which will have a significant impact on this field of study were published by Snyder, et al. (1978, 1980) and were based on a long term exposure protocol in which Sprague-Dawley rats and AKR/J and C57BL/6J mice were exposed to benzene at 100 ppm or 300 ppm. In the first report they demonstrated that Sprague-Dawley rats exposed to benzene at 300 ppm for 6 h/d, 5 d/w exhibited lymphocytopenia, mild anemia and a slight decrease in survival time. AKR/J mice had severe lymphocytopenia and anemia along with granulocytosis and reticulocytosis. They did not grow as rapidly as controls and did not survive as long. A comparison of AKR/J and C57BL/6J mice exposed at either 100 or 300 ppm for life showed that anemia and lymphocytopenia occurred in AKR mice and 20% of the exposed animals developed marrow hypoplasis. Anemia,

TABLE 7.

SUMMARY OF BENZENE INHALATION TERATOLOGY

Study	Species	Strain	Inhalation —7 Exposure Study (ppm)	Duration	Decreased Material Body Weight	Decreased Total Body Weight	Decreased Crown Rump Distance	Comments or Observations
Hazelton, 1975 (as cited in Murray, et al. 1979)	Rat	Sprague-Dawley	0	Day 6 to day 16 of gestation	--	--	--	--
			10		--	--	--	--
			50		*(a)	--	--	--
			500			--	*	Malformations[b]
Green. et al. 1978	Rat	Sprague-Dawley	100	Day 6 to day to 16 of gestation	--	--	--	Missing sternebra*
			300		--	--	--	Missing sternebra* (most in females)
			2,200		*	*	*	Missing sternebra*
Murray, et al. 1979	Mouse	CF-1	500	Day 6 to day 18 of gestation	--	*	--	Missing sternebra* Delayed skull ossification*; unfused occipital*
	Rabbit	New Zealand	500	Day 6 to day 18 of gestation	--	--	--	Extra ribs*; lumbar spur(s)*

(a) * = Statistically significant ($p < 0.05$)

(b) Exencephaly, angulated ribs, out-of-sequence ossification of forefeet

VII. HEALTH EFFECTS IN HUMANS

A. Acute and Chronic Toxicity

Short term exposure to relatively high levels of benzene primarily produce central nervous system effects. Such include dizziness giddiness, exhiliration, nausea, vomiting, headache, drowsiness, staggering, loss of balance, narcosis, coma, and death.

The level at which central nervous system effects will occur in man is not clear from the literature. Hamilton (1931) reviews the earlier literature on acute benzene poisoning, including nineteenth century autopsy findings, and notes a few instances in which central nervous system effects apparently persisted for at least 12 days following a severe initial acute poisoning episode. Gerarde (1960) provides a table summarizing acute effects in which it is stated that 19,000-20,000 ppm for 5-10 minutes is fatal benzene level; 7,500 ppm for 30 minutes is dangerous to life; 1,500 ppm for 60 minutes provides serious symptons; 500 ppm for 60 minutes leads to symptoms of illness; 50-150 ppm for five hours produces headache, lassitude, and weakness, and 25 ppm for 8 hours has no effect. The NAS review (1976) states that exposure in the region of 25,000 ppm is rapidly fatal.

It should be emphasized that mild central nervous system effects appear to be rapidly reversible following cessation of exposure. There is no evidence that they result in chronic brain damage. Also of importance is that these effects appear to be

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concentration-dependent. Lower levels of benzene do not seem to elicit these responses no matter how long the exposure.

Numerous recent reviews evaluating various aspects of benezene toxicology have appeared. These include articles by academic scientists in the open literature (Goldstein, 1977; Snyder and Kocsis, 1975; Snyder, et al., 1977), efforts by scientific bodies such as the International Agency for Research on Cancer (1974), the National Academy of Sciences (1976) and the National Cancer Institute (1977), and reviews performed as part of the regulatory process (NIOSH, 1974; NIOSH, 1977). Among the latter are two previous efforts by the Environmental Protection Agency: "Assessment of Health Effects of Benzene Germane to Low-Level Exposure," published by the Office of Health and Ecological Effects in September 1978, and a document entitled "Ambient Water Quality Criteria for Benzene," released by the Office of Water Regulations and Standards, Criteria and Standards Division in October 1980.

Benzene has been a known hematological poison since the 19th century when Santesson (1897) described cases of aplastic anemia in workers fabricating bicycle tires. The original association of acute leukemia with benzene exposure was made in 1928 (Delore and Borgomano, 1928) and there is a reasonable likelihood that benzene is a cause of acute myeloblastic leukemia. Other hematological diseases have also been reported to be associated with benzene exposure. The major question about the hematological effects of benzene pertinent to the regulatory process is the dose at which they occur. The difficulties in arriving at firm decisions concerning the dose of benzene responsible for adverse effects are in part complicated by a lack of understanding of the pathogenesis of benzene induced hematological disorders, and in particular the relationship between aplastic anemia and acute myelogenous leukemia.

Aplastic Anemia; Pancytopenia. Aplastic anemia is a relatively rare, often fatal, disorder in man. Its diagnosis is usually made on the basis of a significant reduction in the formed elements of the blood: including a decreased white blood cell count known as leukopenia, a decreased red blood cell count known as anemia, and a decreased platelet count known as thromboycytopenia. A decrease in all three of these blood cell counts is descriptively defined as pancytopenia. Only the more severe cases, which are generally associated with a marked decrease in the number of cells in the bone marrow, are usually called aplastic anemia. The important point is that these are not distinct diseases but rather a continuum of changes reflecting the severity of bone marrow toxicity due to benzene toxicity. This is demonstrated by studies in which following the observation of one severely affected benezene-exposed worker, complete evaluation of the work force revealed many other affected individuals with effects ranging from a mild individual cytopenia to aplastic anemia of sufficient severity to warrant hospitalization.

Among the studies which have shown this wide range of hematological response are those of Goldwater and his colleagues (Goldwater, 1941; Goldwater and Tewksbury, 1941; Greenburg, et al., 1939) evaluating over 300 rotogravure printers in New York. A change in the printing process had led to the exposure of these workers to levels of benzene described as ranging from 11 to 1,060 ppm. There were 23 cases of significant cytopenias, 6 of whom required hospitalization. Wilson in 1942 reported studies of 1,104 workers in a rubber factory in Ohio who were exposed to up to 500 ppm benzene with an average of about 100 ppm. Mild hematological abnormalities were noted in 83, more severe pancytopenia in 25 and 9 of the latter were hospitalized, 3 of whom died. Savilahti (1956) reported that 107 of 147 Finnish shoe factor· workers were noted to have some hematological abnormalities. Concentrations of benzene, which had been in use for about 10 years, were as high as 400 ppm. Of note is that Hernberg, et al. (1966) performed a follow-up study of 125 of these workers 9 years later. They noted some persistent cytopenias. One individual had developed acute leukemia and died. Another study with a long follow-up is that of Guberan and Kocher (1971). They followed 216 of 282 workers for 10 years after cessation of benzene exposure. Four individuals are reported to have persistent decrease in blood counts and one patient had died of aplastic anemia 9 years after cessation of exposure. Follow-up data suggesting mild persistent anemia workers in the rubber coating industry were presented by NIOSH (1974). These individuals had been exposed to benzene levels described as generally less than 25 ppm but ranging up to 125 ppm prior to installation of control measures (Pagnotto, et al., 1961).

Other large series of cases of aplastic anemia in benzene-exposed work groups have been provided by Vigliani and his colleagues in Italy, although these investigators have focused primarily on leukemia (Vigliani and Forni, 1976; Saita and Vigliani, 1962), and the studies of Aksoy, et al., in Turkey (1971, 1972a, and 1978). In the latter, an outbreak of hematological toxicity in leather workers was directly temporally related to the use of an adhesive containing benzene beginning in about 1960. Aksoy, et al. (1972a) reported on 32 cases of significant aplastic anemia in people exposed to benzene for four months to fifteen years. Exposure levels ranging from 150 to 650 ppm were reported. In another study reported by this group (Aksoy, et al., 1971) 51 of 217 apparently healthy workers were found to have some hematological abnormalities, including 6 cases of pancytopenia. These workers are described to have been exposed to 30 to 210 ppm benzene, for 3 months to 17 years. It should be noted that the exposure levels reported in this series of studies represent occasional random measurements of what is in essence a cottage industry.

These studies of occupationally exposed groups are notable for the association of benzene with pancytopenia in workers from different countries and in different work settings where the only common denominator appears to have been benzene. The clear temporal relationship between the onset and cessation of hematological abnormalities and the use of benzene provides further evidence allowing the conclusion that a causal relationship between benzene exposure and pancytopenia is incontrovertible. This is further substantiated by the ability to reproduce these findings in different animal species (see Section V).

None of the above occupational studies, however, provides information concerning the lowest dose of benzene that might be expected to produce cytopenic affects in man. Two studies of occupationally exposed groups which do attempt to provide information on the subject are by Doskin (1971) from the Soviet Union and Chang (1972) from Korea. These studies in the non-English literature are described in some detail in the "Assessment of Health Effects of Benzene Germane to Low-Level Exposure" (EPA, 1978). In this assessment, it is emphasized that many details as to exposure are not provided by Doskin or by Chang. Chang studied 119 workers exposed to Benzene in an unspecified industrial area. Hematological abnormalities were observed in 28: including 21 with anemia, 2 with leukopenia, and 5 with both. The author plots a graph in which each of these affected individuals is characterized by duration of work on the abscissa and level of benzene exposure on the ordinate. Based on this plot, the author obtained an exponential function that implied a "threshold" of 10.0 ppm benzene for cytopenic effects. However, no hematological toxicity was observed in the 18 workers exposed to 10 to 20 ppm benzene. A major problem in interpreting this study is the absence of information concerning the definition of work exposure concentrations for the individual employees.

Doskin (1971) in the Soviet Union evaluated 365 individuals employed for three years in what was apparently a new chemical factory. Serial hematological studies were performed on the exposed workers as well as the control group. Benzene exposure levels are given in terms of the maximum permissible concentration which apparently was 5 ppm. It is noted that benzene levels exceeded this minimum permissible concentration two to eight-fold in 64% of the measurements in the first year, 37% in the second year, and 3% in the third year. This decrease in benzene levels paralleled a decrease in the number of workers who had hematological abnormalities. In the first year close to 40% of the workers exhibited mild hematological abnormalities, the most common being thromboycytopenia (95-155,000/mm^3). Inasmuch as the maximal permissible concentration in the Soviet Union at the time of the study was apparently 5 ppm, these findings suggest that exposure of workers to concentrations of 10 to 40 ppm benzene for less than one year produces mild cytopenic effects. Interpretation of this study would be furthered by information concerning the benzene monitoring system and the actual levels recorded.

The qualitative abnormality that has received the most attention in association with benzene exposure is that of cytogenetic changes in the nucleus of bone marrow cells or of circulating lymphocytes of exposed individuals. Numerous case reports in individuals with clear-cut hematological toxicity in association with benzene-exposure have been well reviewed elsewhere (Wolman, 1977; EPA 1978). Studies of occupationally exposed groups include the work of Forni, et al. (1971a) who compared cytogenetic findings findings in 34 workers at a rotogravure plant with those of matched controls. Exposure was to benzene alone, or benzene plus toluene, with the benzene levels ranging well over 100 ppm. Workers exposed to toluene alone were not different from the age and sex matched controls. However, there was statistically significanct increase in chromosomal abnormalities in the benzene exposed group. This group also studied 25 individuals who had recovered from benzene hematotoxicity (Forni, et al., 1971b). There was a tendency toward a decrease in unstable chromosome changes over time. However, a persistence or an increase in stable chromosome changes was generally noted.

Tough and Court-Brown (1965) observed significant chromosomal damage in cultured lymphocytes from workers exposed to benzene. In a further study (Tough, et al., 1970) benzene-exposed workers from three separate factories were studied along with control individuals from the same workplace. Unstable chromosomal abberrations were observed in exposed workers but not in control workers from a factory in which benzene levels were recorded as 25 to 150 ppm. In another factory with similar benzene concentrations unstable abberrations were found in both control and exposed workers, but in neither group in a third factory in which benzene levels were recorded as approximately 12 ppm.

Certain of the qualitative changes in blood cell that are described above would clearly be considered as adverse effects from

from which the public should be protected. Cytogenetic abnormalities would appear to fall into this category in that they have been associated with carcinogenesis, although absolute direct proof of such a relationship in man has not been forthcoming. On the other hand, it is difficult to assign clinical importance to small changes within the normal range of such parameters as serum immunoglobulins.

Acute Myeloblastic Leukemia. Acute myeloblastic leukemia is a cancer in which there is an abnormal proliferation of the hematologic precursor cell which is believed to be the common progenitor for granulocytic leukocytes, red blood cells and platelets. This disease is mostly observed in adults and has an increasing incidence with age, peaking in the sixth or seventh decade. There are a number of variants of acute myelogenous leukemia which, for purposes of the present discussion, can be considered to be part of the same disease. These include acute myelomonocytic leukemia, promyelocytic leukemia, and erythroleukemia.

Since the original report of Delore and Borgomano (1928) there have been well over a hundred individual cases of acute myeloblastic leukemia or its variants in which an association with benzene exposure has been reported. Obviously, the most any one case report can do is to suggest an association. The credence that these case reports give to the causal relation of benzene to acute myelogenous leukemia only in part reflects the number of cases. Particularly impressive is the relatively common report of an individual with aplastic anemia associated with benzene exposure who is followed through a preleukemic phase into frank acute leukemia (Aksoy et al., 1972b; 1976; Girard and Revol, 1970; Mallein et al., 1971; Tareef et al., 1963). Also of note is the frequency with which erythroleukemia is reported in association with benzene exposure. This is a rare variant of actue myelogenous leukemia.

Also of note in the case reports is the sometimes long delay between the cessation of known benzene exposure and the onset of acute leukemia (Aksoy et al., 1972b; DeGowin, 1963; Guasch et al., 1959; Justin-Besancon et al., 1959; Ludwig and Werthemann, 1962; Robustelli della Cuna et al., 1972; Saita and Vigliani, 1962; Sellyei and Keleman, 1971; Vigliani and Saita, 1964). Among these cases is that of DeGowin who noted a painter with acute leukemia 15 years after the diagnosis of a benzene-related aplastic anemia. An even longer interval was reported by Justin-Besancon et al. (1959) who observed a case of acute leukemia 27 years after exposure to benzene. Chromosomal abnormalities and Pelger-Huet anomaly were reported in a case of acute granulocytic leukemia which occurred seven years after benzene-induced pancytopenia (Sellyei and Keleman, 1971). One of the cases in the series of Vigliani and Saita (1964) occurred 12 years after cessation of occupational benzene exposure. Guasch, et al. (1959) reported a case of acute myelogenous leukemia 6 years after onset of pancytopenia and also provided a relatively extensive review of earlier case reports.

Taken together these individual case reports provide relatively strong circumstantial evidence of a causal relationship between benzene exposure and acute myeloblastic leukemia. In addition to the points raised above, among the more convincing aspects of this assemblage of case reports is that they come from all over the world and represent diverse occupational exposure settings which have in common substantial exposure to benzene.

B. Epidemiology

Formal epidemiologic methods have been used in studying the relationship between exposure to benzene and the development of leukemia. The studies have concentrated on industrial exposure to benzene. Because it is rare for exposure to any industrial chemical to occur in isolation, interpretation of these studies must take into account that the excess occurrence of leukemia (or of other diseases) may be due to confounding by other substances.

This section is divided arbitrarily into reports where there was a reasonable likelihood that there was exposure to benzene and reports where exposure to benzene either was uncertain or was complicated by exposure to other solvents.

Studies of Persons Exposed to Benzene. Aplastic anemia as a chronic effect of benzene exposure was recognized clinically because it developed among persons who used solvents containing a high proportion of benzene (25%+). Because some of the persons with aplastic anemia eventually developed acute myeloblastic leukemia, it was natural to conclude that high exposure to benzene caused leukemia. However, epidemiologists evaluate the possibility that relatively short-term, low-level exposure to benzene leads only to leukemia, but not to aplastic anemia.

The studies of Aksoy and his colleagues in Turkey provide a bridge between the clinical description of leukemia in persons exposed to benzene and the epidemiologic evaluation of that association (Aksoy _et al_., 1966-1977). Between 1955 and 1960 a solvent containing high levels of benzene was introduced into the Turkish shoe industry (Aksoy and Erdem, 1978). In 1961 cases of aplastic anemia were observed among these workers and cases of leukemia appeared in 1967.

In 1974, Aksoy, _et al_. (1974b) estimated the incidence rate of acute leukemia or preleukemia among shoe workers. Between 1967-73, 26 workers who had been exposed to benzene were admitted to Turkish hospitals. On the basis of official records, Aksoy estimated that there were 28,500 workers involved in the shoe, slipper, and handbag industry in which benzene is used as a solvent. Assuming a seven-year follow-up period, the incidence rate of leukemia was then 26/(28,500 x 7) = 28/199,500 person-years = 13/100,000/year. Aksoy compared this to an estimated incidence rate of 6/100,000/year in the general population.

Because of methodologic shortcomings, these data of Aksoy _et al_., are difficult to interpret:

1. The definition of occupation used for the person with leukemia differed from that used for the "official

records." It is possible that a person with leukemia was called a shoeworker whereas the same person appeared on the official record as having another occupation;

2. No follow-up of the 28,500 workers with exposure to benzene was carried out. It is possible that all cases of leukemia were not ascertained;
3. The comparison of incidence rate of 6/100,000/year is for an unknown location and an unknown time;
4. No age-standardization was done.

In a second report, Aksoy followed 44 pancytopenic patients who had had chronic exposure to benzene. Six of these patients developed leukemia of unspecified type. Five of these six patients had pancytopenia at the time leukemia was diagnosed. Thus, a question can be raised as to the chronology of the diagnostic process. While there was no formal comparison of the rate of leukemia in another group, 6/44 is a high proportion on grounds of common knowledge.

Another bridge between clinical and epidemiologic evaluation of benzene is in the report of Hernberg, _et_ _al_. (1966). In 1955, 149 persons in a Finnish shoe factory were heavily exposed to benzene and more than 100 had blood abnormalities. In a follow-up in 1964, one person had been identified as having developed leukemia. While the number that might have been expected in an unexposed group was not estimated, it is clear that any estimate of excess risk or absence of such risk would be very unstable.

Thorpe (1974) evaluated leukemia mortality among petroleum workers who had potential exposure to benzene as a constituent of petroleum products. Among eight affiliates of Exxon, 18 cases of leukemia were observed over a 10-year period. On the basis of WHO age-specific mortality rates for leukemia, 23.2 deaths were expected. The data in this study are difficult to interpret because of an uncertainty as to actual level of exposure to benzene and because it is not clear that most cases of leukemia in the study population were ascertained.

Infante, *et al*. (1977a,b) provided data from a retrospective cohort study of workers at 2 "Pliofilm" production plants in Ohio. Pliofilm was a product made from natural rubber suspended in a solvent solution containing benzene. While actual measurements of benzene exposure were not available until the early 1960's, levels between 1945 and 1975 were believed to be generally within the contemporary standard (100 ppm in 1941 to 10 ppm in 1971). Mortality between 1/1/50 and 6/30/75 was determined for 748 workers employed between 1/1/40 and 12/31/49. There were 140 deaths from all cases observed and 187.6 expected on the basis of age-time specific deaths for U.S. white males. There were seven deaths observed from leukemia in comparison to 1.4 expected on the basis of U.S. white male mortality rates or 1.5 expected on the basis of mortality rates from a comparison cohort of fibrous glass workers ($p < 0.002$). Of the seven leukemias, four were acute myelogenous, two were monocytic and one was chronic myelogenous.

Rinsky, et al., published a follow-up study to the Infante (1977) study. Rinsky used the idential cohort. His report was based upon 98% vital status follow-up vs. 75% for the Infante study. Among 748 workers who had at least one day of exposure to benzene between 1940 and 1950, seven deaths due to leukemia occurred. In the United States the death rates standardized for age, sex and calendar time period, was expected to be only 1.25 leukemia deaths. The standardized mortality ratio (SMR) is equal to 560; $p < 0.001$. The mean duration of benzene exposure was very brief and 437 (58%) of the cohort were exposed for less than 1 year. The evaluation of exposure to benzene of workers exposed five or more years showed leukemia deaths that produce an SMR of 2,100. The leukemia cell types were myelocytic or monocytic. Four additional cases of leukemia were recognized but not included for technical reasons. Had they been included in the cohort, the SMR would equal 3,780. Rinsky reconstructed the past exposure to benzene at the two locations. The analysis indicated that in some areas of the plant airborne benzene concentrations rose occasionally to several hundred parts per million (ppm), but for the most part, employee eight-hour time-weighted averages (TWA) fell within the limits considered permissible at the time of exposure. These data indicate that benzene is a human carcinogen at levels not greatly above the current legal standard.

This study was critized by Tabershaw and Lamm (1977). They state that some exposed groups at the two plants were not included and that this report was a rediscovered cluster of leukemia.

These criticisms were answered by the authors; specifically, they state that the study cohort was selected before they had any knowledge of the occurrence of leukemia.

This study provides the strongest epidemiologic evidence of an excess of leukemia among persons exposed to benzene. However, because of uncertainties as to the level of exposure and the generalizability of these data, this one study alone cannot be regarded as definitive.

Ott, et al. (1978) and Townsend, et al. (1978) reported mortality and health exam findings among individuals occupationally exposed to benzene at the Michigan Division of Dow Chemical. In the mortality study, 594 workers involved in three production areas on or after 1/1/40 through 1/1/74. Between 1953 and 1972 the time weighted-average exposure to benzene was estimated to be generally less than 10 parts per million, although there were some exposures above 30 ppm. Among the entire population 102 deaths were observed and 128.2 were expected on the basis of age-time specific death rates for U.S. white males. Two deaths from leukemia (leukemia, acute myelogenous leukemia) were observed and 1.0 were expected. A third person's death was attributed to pneumonia but the person had acute myeloblastic leukemia. These data are too few to provide an independent stable estimate of the relation between benzene and leukemia.

In the report on health exam findings among 282 of this cohort, Townsend, et al. (1978) concluded that there was no indication that adverse effects of benzene had occurred in their cohort.

Studies of Persons with Possible Exposure to Benzene. In a proportional mortality analysis of cause of death among 3,637 chemists, Li, et al. (1969) noted an excess of pancreas cancer (36 observed, 22 expected) and of lymphatic and hematopoietic cancer (94 observed, 59 expected). Included were 33 death observed from leukemia in comparison to 25 expected. While it might be expected that as a group chemists would have more exposure to benzene than would the general population, no information on exposure was available.

In a case-control study of leukemia in Japan, Ishimaru, et al. (1971) studied 303 matched pairs of leukemia cases and their controls, 30 of the cases and 124 of the controls had had potential occupational exposure to jobs in which benzene and/or medical X-ray exposure was likely. However, no information was obtained on specific chemical agents, and it is speculative to attribute the excess among the cases to benzene exposure.

In 1972 Redmond, et al., evaluated mortality among 4,661 coke oven workers in 12 steel plants in the United States. Benzene is one of the side products of coke production. Among all workers, nine deaths from leukemia were observed and 10.8 were expected.

From 1976 through 1978, a number of reports have been published on martality among U.S. rubber workers. (McMichael, et al., 1974, 1975, 1976a, 1976b; Andejelkovich, et al., 1976, 1977; Monson and Nakano, 1976a, 1976b; Monson and Fine, 1978). A number of solvents, including benzene, have been used in the production of rubber products. In many of these reports there has been an excess occurrence of leukemia, both myelogenous and lymphatic. While the excess tended to occur in areas with relatively high solvent exposure, no estimates of exposure to benzene were available.

Greene, et al., reported in 1979 the proportional cancer mortality experience of 347 male employees of the U.S. Government Printing Office. Benzene had been used at this facility only on a limited basis, primarily in the bindery. There were 16 deaths from leukemia observed and 11.6 expected overall. Among binders, there were 5 deaths from leukemia observed and 2.8 expected. Among white binding workers there was a significantly elevated population mortality ratio of 315.

Vianna and Polan (1979) reported an excess occurrence of lymphoma among a number of occupations where benzene and/or coal tar fractions are used. Based on a stable number of deaths in New York State, from 1950 through 1969, crude relative risks based on mortality rates were: reticulum cell sarcoma--1.6, lymphosarcoma--2.1, and Hodgkin's disease--1.6. These data share the limitations of the Aksoy study in that occupation for the denominator of the rate was estimated on the basis of census data and occupation for the numerator was determined from the death certificate. Further, no estimate of actual benzene exposure was possible.

VIII. MECHANISM OF TOXICITY

The early studies on the mechanism of benzene-induced bone marrow depression evaluated bone marrow and for the most part emphasized morphology. The classical studies of Selling (1916) included a description of rabbit bone marrow following chronic intoxication with subcutaneously administered benzene. The first signs of bone marrow damage were evident on the second day of injection with 1 mL/kg and by the ninth day aplasia was complete. All of the cell types were damaged and gradually disappeared during the course of treatment. Considerable evidence has developed to support the concept that benzene produces its effect by inhibiting cell division. Fewer mitotic figures were observed in the marrow of benzene intoxicated animals and benzene has been shown to cause abnormal mitotic figures (Pollini, et al., 1965). Recently Sammett, et al. (1979) demonstrated that livers of partially hepatectomized rats failed to grow back when the animals were treated with benzene, and D'Souza, et al. (1979) reported that the remaining ovary in the hemi-spayed rat did not undergo compensatory cell proliferation after treatment with benzene. These data suggest that benzene inhibited cell proliferation. Thus, it may be that the reason that benzene does not act as a primary liver toxin but is highly effective against the bone marrow is that liver cells do not normally undergo rapid cell proliferation whereras rapid proliferation is a property of bone marrow cells.

The effects of benzene on chromosomes will be discussed under the section on the mutagenic potential of benzene but some reference to these effects is important here because they reflect on mechanisms of cell replication. Chromosome aberrations following benzene treatment or exposure have been observed by Kissling and Speck (1969, 1972) Forni, *et al.* (1971a,b), and Tough, *et al.* (1970). Although cellular damage which results in mitotic arrest is readily observed, it is not clear where the initial attack occurred. Thus, Moeschlin and Speck (1967) and Kissling and Speck (1969, 1972) reported decreases in bone marrow uptake of tritiated thymidine into DNA and tritiated cytosine into RNA after treating rabbits with benzene subcutaneously. They claimed that benzene inhibited the synthesis of both types of nucleic acid. Boje, *et al.* (1970) made similar observations after exposing rats to benzene by inhalation but suggested that the decrease may have been explained by a number of effects such as degradation of nucleic acids, reutilization of tritiated thymidine, changes in pool size or alteration in the cell cycle. These observations require further clarification.

The postulate that a metabolite of benzene mediates benzene-induced hemopoietic toxicity is supported by several lines of evidence. Studies in the rat, rabbit and dog have indicated that decreased metabolism of benzene correlates with decreased toxicity. Hirokawa and Nomiyama (1962) and Nomiyama (1962) have shown that rats whose livers oxidized benzene less rapidly *in vitro* were less susceptible to benzene poisoning. Abramova and Gadakina (1965) showed that the administration of antioxidants such as propyl gallate, cystine, cysteamine and methionine to rabbits decreased benzene metabolism in vivo and in vitro and also decreased the leukopenia seen after chronic benzene administration. Hough and Freeman (1944) reported that dogs exposed to a mixture of benzene, toluene and xylene metabolized less benzene, had higher leukocyte counts and survived longer than dogs exposed to benzene alone. Each of these studies concluded that a decrease in benzene metabolism is accompanied by a decrease in benzene-induced hemopoietic toxicity.

Andrews, et al. (1977a) administered ^{3}H-benzene to mice (440 or 880 mg/kg) subcutaneously with or without toluene (2mL/kg) a competitive inhibitor of benzene metabolism. ^{3}H-benzene and its metabolites were measured in urine and tissues of the mice, and toxicity was measured using the ^{59}Fe uptake method of Lee, et al. (1974). Co-administration of toulene with benzene rendered benzene less effective as an inhibitor of red cell ^{59}Fe uptake and reduced the accumulation of benzene metabolites in urine. A 2:1 ratio of toluene to benzene partially alleviated benzene toxicity, whereas a 4:1 ratio of toluene to benzene completely prevented benzene toxicity. The protective effect of toluene appears to result from the inhibition of the conversion of benzene to a toxic metabolite.

A striking observation in this experiment was that levels of benzene metabolites in bone marrow far exceeded levels in all other tissues. Peak metabolite levels in bone marrow reached a level of 900 nmoles/g wet weight but only 110 nmoles/g in blood. The accumulation and persistence of such high levels of metabolites in bone marrow suggest the possibility that it is these metabolites, rather than the covalently bound metabolites, that cause the initial depression in bone marrow function. The accumulation of benzene metabolites in bone marrow was apparently not due to the uptake of metabolites from blood, since ^{3}H-phenylsulfate, ^{3}H-phenylglucuronide and ^{3}H-phenol did not concentrate in bone marrow after iv injection. It was postulated, therefore, that the metabolites may be formed and sequestered within bone marrow.

Rickert, et al. (1979) described the distribution of benzene and its metabolites in rats exposed to 500 ppm benezene in the air. In this experiment, levels of free phenol, catechol and hydroquinone were detected in blood and bone marrow. Furthermore, levels of phenolic metabolites in bone marrow exceeded the respective levels in blood. These results paralleled those of Andrews, et al. (1977a) who administered benzene subcutaneously to mice. Therefore, the distribution of benzene metabolites was essentially the same whether benzene was given via inhalation or by subcutaneous injection.

Not all experiments, however, have demonstrated the ~~con-~~ concentration of benzene metabolites in bone marrow. Bergman (1979) used whole body autoradiographic techniques to localize ^{14}C-benzene and its metabolites in tissues of mice exposed by inhalation. The amount of ^{14}C-benzene was determined by low temperature autoradiography and the amount of ^{14}C-benzene metabolites by autoradiography of dried, evaporated sections. No selective accumulation of benzene metabolites was found in bone marrow. These results are difficulat to rationalize in terms of the results of Andrews, et al. (1977a) and Rickert, et al. (1979). However, Andrews, et al. (1977a) determined benzene metabolites as conjugates of phenols and Rickert, et al. (1979) determined metabolites by a gas-liquid chromatography mass-spectrometric technique. These techniques are more specific and analytical than whole body autoradiography. Additionally, Greenlee, et al. (1980), using radioautographic techniques, have recently observed that pyrocatechol and hydroquinone, which are known metabolites of benzene, concentrate in hemopoietic organs of the rat.

Recent studies using the ^{59}Fe uptake technique to evaluate benzene toxicity have added to the evidence linking benzene metabolism with its toxicity. Longacre, et al. (1980) demonstrated a correlation between benzene metabolism and hemopoietic toxicity in several strains of mice. In these experiments male CD-1, C57/B6 and DBA/2 mice were given ^{3}H-benzene and both the red cell ^{59}Fe uptake and the levels of benzene metabolites in urine and tissues were determined. A comparison between the most sensitive strain, the DBA/2, and the least sensitive strain, the C57/B6, revealed that the levels of benzene metabolites in the bone marrow and other organs were higher in the more sensitive animals. Another experiment demonstrated the primary role of the liver in benzene toxicity (Sammett, et al., 1979). Sprague-Dawley rats that had undergone partial hepatectomy (70-80% of the liver was removed) were given ^{3}H-benzene, and benzene metabolism and toxicity were measured.

Partial hepatectomy decreased the metabolism of benzene by 70% and completely protected against benzene-induced hemopoietic toxicity when compared to sham-operated animals that received benzene.

One explanation for the presence of high concentrations of benzene metabolites in bone marrow is that these metabolites are formed in bone marrow and thereby accumulate in that tissue. Andrews, et al. (1977b) have shown that subcellular fractions from rabbit bone marrow contain benzo(a)pyrene hydroxylase, a mixed-function oxidase enzyme. Benzo(a)pyrene hydroxylase activity is highest in the microsomal fraction, was inducible as a result of treatment of rabbits with 3-methylcholanthrene and was inhibited by low concentrations of 7,8-benzoflavone. More recently, hydroxylation of benzene to phenol has been shown to occur in rabbit bone marrow *in vitro* (Andrews, et al., 1979). In these experiments, ^{3}H-benzene metabolism was determined in the microsomal fraction of rabbit femoral bone marrow. Cytochrome P-450 (26-51 pmoles/mg microsomal protein) were detected in bone marrow microsomes. ^{3}H-benzene metabolism (2 pmoles benzene equivalents/mg microsomal protein/min) required the presence of an NADPH-generating system and was inhibited 80% in the presence of a $CO:O_2$ (9:1) atmosphere. The products of benzene metabolism were phenol and an unknown metabolite.

Recently, Irons, et al. (1980) have described the metabolism of benzene in rat bone marrow that was perfused *in situ*. The left common iliac artery and vein were cannulated, the isolated limb was perfused with whole rat blood and ^{14}C-benzene was introduced through the head of the femur into the marrow space. Blood was recirculated and collected at successive time intervals. Bone marrow was collected at the end of the experiment for determination of metabolites. Radiolabeled phenol, catechol and hydroquinone were isolated from both blood and bone marrow. In these studies benzene metabolites accumulated in the marrow much as they do _in vivo_. The rate of benzene metabolism in marrow is much lower than liver, an observation probably related to the low level of mixed-function oxidase activity found in bone marrow.

Despite evidence showing that benzene can be metabolized in bone marrow, production of toxic metabolites of benzene by bone marrow may be insufficient to produce bone marrow depression. Sammett, _et al_. (1979) recently demonstrated that partial hepatectomy protected rats and mice from benzene-induced depression of erythropoiesis. The protective effect was accompanied by a decrease in urinary levels of benzene metabolites, a reduction of soluble benzene metabolites in bone marrow and a lowering of covalent binding of reactive metabolites of benzene to bone marrow protein. Although bone marrow can metabolize benzene, apparently benzene metabolism in the liver plays a more important role in the development of bone marrow toxicity. These previous reports of protection against toxicity in phenobarbital-treated animals (Ikeda and Ohtsuji, 1971; Drew and Fouts, 1974) reflect the fact that phenobarbital probably increased the detoxification rate in liver. On the other hand, inhibition of metabolism by toluene and also by aminotriazole (Hirokawa and Nomiyama, 1962) protected animals by decreasing the rate of formation of toxic metabolites. Thus, it appears that a metabolite formed in liver is transported into the marrow where it is converted to a compound which cannot be removed from the marrow and accordingly accumulates (Andrews, et al., 1977b; Rickert, et al., 1979) leading to a metabolic impairment expressed as bone marrow depression. Similar mechanisms may play a role in benzene-induced leukemogenesis.

IX. RISK ASSESSMENT

It is not intended here to develop a new risk assessment for benzene. This section will report and comment on the existing literature as it relates to the development of risk assessments for benzene.

Benzene holds a unique position in industrial chemistry because of its extensive use for more than 100 years and the fact that new uses are continually developing. Its first heavy use was in the 19th century (Hunter, 1962) in the rubber industry where it was an excellent solvent for rubber latex. Some of the first cases of benzene poisoning were observed among women who painted benzene-based rubber cement on cans to seal the lids (Selling, 1910). In each, upon evaporation of the benzene the hardened rubber remained and provided either molded rubber products, rubberized fabrics, or rubber seals for cans. Benzene poisoning was also observed extensively in the rotogravure printing industry in this country (Mallory, 1939) because it was used as a rapidly drying solvent for the various colored inks in high speed printing presses. Almost any process that has required a good aromatic solvent has made use of benzene because of its properties plus its low cost.

In addition to its use as a solvent, benzene has been used extensively as a starting material in chemical syntheses. Following World War II with the development of the plastics industry, benzene utilization increased dramatically because it serves as a source for the synthesis of the monomeric units of polystyrene plastics. The total amount of benzene used is controlled by the availability of resources for its manufacture (Anonymous, 1973). It ranks with ethylene and propylene, also precursors to plastics, as the most heavily produced organic chemicals in the world. Other major uses of benzene have been described by Fick (1976) who showed that benzene was essential for the production of ethylbenzene (an intermediate in styrene production) cumene, cyclohexane, aniline, maleic anhydride and numerous other compounds made in smaller quantities. It is also added to gasoline as part of the aromatic fraction which is replacing alkyl lead compounds as anti-knock ingredients. It is unlikely that the use of benzene in industry will be abandoned, but the International Workshop on Benzene (Truhaut and Murray, 1978) recommended that all use of benzene as a solvent should be discontinued since there are excellent substitutes available. Thus, the only recommended use of benzene is in chemical syntheses.

Recommendations to limit exposure to benzene in the workplace came soon after the problem of benzene toxicity was recognized. Thus, in 1922, Alice Hamilton, one of the pioneers in industrial medicine, wrote a telling report on "The growing menace of benzene (benzol) poisoning in American industry." In part because of the alarm she raised, attempts to control benzene exposure were instituted on a voluntary basis and she found it possible to report in 1928 on "The lessening menace of benzol poisoning in American industry." The problem continued, however, and the description of extensive benzene poisoning in the rotogravure printing industry in New York (Mallory, et al., 1939) was in part responsible for the establishment of an MAC value (maximum allowable concentration) of benzene in the workplace by the newly formed American Conference on Governmental Industrial Hygienists (ACGIH) in the late 30's-early 40's. ACGIH standards are voluntary and do not carry the force of law. However, attempts to comply with these recommendations were reportedly pursued. In 1946 an MAC of 100 ppm was allowed in the workplace (Zenz, 1978). In 1947 the standard was reduced to 50 ppm as a time weighted average over an eight hour day. Further reduction to a 25 ppm TWA over eight hours was applied in 1948 and a 25 ppm ceiling was added in 1963. Thus, not only was the exposure restricted to an average of 25 ppm over a workday, but by applying a ceiling, no excursion above 25 ppm, even for brief periods, was allowed. In 1970 the TWA was reduced to 10 ppm and by 1974, when OSHA and NIOSH came into the picture, the 10 ppm was complemented with a 25 ppm ceiling (NIOSH, 1974). In 1976, OSHA and NIOSH reached the conclusion that benzene was a leukemogen, and the 10 ppm standard was not considered sufficient to protect the worker. Further, discussion of the OSHA conclusions is provided in the following excerpt from a Supreme Court syllabus (Supreme Court, 1980).

> OSHA noted that there was "no dispute" that certain nonmalignant blood disorders, evidenced by a reduction in the level of red or white cells or platelets in the blood could result from exposures of 25-40 ppm. It then stated that several studies had indicated that relatively slight changes in normal blood values could result from

exposures below 25 ppm and perhaps below 10 ppm. OSHA did not attempt to make any estimate based on these studies of how significant the risk of nonmalignant disease would be at exposures of 10 ppm or less.(a) Rather, it stated that because of the lack of data concerning the linkage between low-level exposures and blood abnormalities it was impossible to construct a dose-response curve at this time.(b) OSHA did conclude, however, that the studies demonstrated that the current 10 ppm exposure limit was inadequate to ensure that no single worker would suffer a nonmalignant blood disorder as a result of benzene exposure. Noting that it is "customary" to set a permissible exposure limit by applying a safety factor of 10-100 to the lowest level by applying a safety factor of 10-100 to the lowest level at which adverse effects had been observed, the Agency stated that the evidence supported the conclusion that the limit should be set at a point "substantially less than 10 ppm" even if benzene's leukemic effects were not considered. 43 Fed. Reg., at 5924-5925. OSHA did not state, however,

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(a) As OSHA itself noted, some blood abnormalities caused by benzene exposure may not have any discernible health effects, while others may lead to significant impairment and even death. 43 Fed. Reg. at 5921.

(b) "A dose-response curve shows the relationship between different exposure levels and the risk of cancer [or any other disease] associated with those exposure levels. Generally, exposure to higher levels carried with it a higher risk, and exposure to lower levels is accompanied by a reduced risk." 581 F.2d, at 504, n.24.

OSHA's comments with respect to the insufficiency of the data were addressed primarily to the lack of data at low exposure levels. OSHA did not discuss whether it was possible to make a rough estimate, based on the more complete epidemiological and animal studies done at higher exposure levels, of the significance of the risks attributable to those levels, nor did it discuss whether it was possible to extrapolate from such estimates to derive a risk estimate for low-level exposures.

(c) OSHA did not invoke the automatic rule of reducing exposures to the lowest limit feasible that it implies to cancer risks. Instead, the Secretary reasoned that prudent health policy merely required that the permissible exposure limit be set". . .

> that the nonmalignant effects of benzene exposure justified a reduction in the permissible exposure limit to 1 ppm.(c)

The result was an extended legal battle in which the American Petroleum Institute, the American Iron and Steel Institute and the Manufacturing Chemists Association prevailed over the Department of Labor in a decision by the Supreme Court. The Court ruled that the rationale for lowering the permissible exposure limit was based not on any findings that leukemia has ever been caused by exposure to 10 ppm of benzene and that it will not be caused by exposure to 1 ppm, but rather on a series of assumptions indicating that some leukemias might result from exposure to 10 ppm and that the number of cases might be reduced by reducing the exposure level to 1 ppm. The court further required that OSHA determine that it is reasonably necessary, appropriate, and feasible to remedy a significant risk of material health impairment. Therefore the standard remains at 10 ppm.

The National Academy of Sciences undertook to report on the benzene problem. In 1977 the Committee on Toxicology of the NAS reviewed the health effects of benzene, most of which have been cited in this report, and recommended that further research

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sufficiently below the levels at which adverse effects have been observed to assure adequate protection for all exposed employees." 43 Fed. Reg., at 5925. While OSHA concluded that application of this rule would lead to an exposure limit "substantially less than 10 ppm," it did not state either what exposure level it considered to present a significant risk of harm or what safety factor should be applied to that level to establish a permissible exposure limit.

was needed to clarify the mechanisms of benzene-induced diseases. These sentiments were echoed in the report of the Safe Drinking Water Committee of the Academy in 1977, when they quoted a figure of 10 ug/L of benzene as the highest observed concentrations in finished water but failed to estimate the upper 95% confidence estimate of lifetime cancer risk per ug/mL for lack of sufficient data.

The U.S. Environmental Protection Agency prepared three documents on the health effects of benzene which were concerned with an assessment of health effects, an assessment of environmental exposure and a population risk assessment. The first (USEPA, 1978) was a complete review of the metabolism, cytogenetic and embryonic effects; a review of the chronic toxicity of benzene in animals; and a review of the pancytopenic, leukemogenic and other toxic effects of benzene in man. Briefly, the conclusions indicated that benzene was leukemogenic and pancytopenic in man and caused genetic damage. There was doubt that the available data could be used to derive a dose-response curve since most evaluations of exposure in the face of benzene toxicity indicated high doses but documentation of benzene toxicity at low doses was poor.

The second, a contractor report prepared for the USEPA by Mara and Lee (1978), was a document on human exposures. The report suggests that although there is clearly human exposure to benzene in water, food and in some cases, soil, exposure via these routes is minimal and the main exposure pathway was considered to be via the air. Sources of benzene included chemical manufacturing plants, coke ovens, gasoline service stations, petroleum refineries, solvent operations, storage, and distribution centers for benzene and gasoline, and exhausts from automobile emissions in urban areas. Because of the paucity of data and the fact that most of it was collected at specific points, it was necessary to make a variety of assumptions with respect to potential human exposure. One involved the use of dispersion models. Estimates were made of the number of people exposed to the lowest measurable concentrations of benzene, namely, 0.1 ppb. A number of uncertainty factors were cited which clearly affected the accuracy of the modeling results. Nevertheless, the report estimated that the results were not in error by a factor greater than an order of magnitude. The sources which lead to most human exposure were reported to be gasoline service stations and urban exposure to auto emissions. It was estimated that 87,000,000 people living in the vicinity of gasoline stations may be exposed to benzene at levels of 0.1-1.0 ppb as an annual average with the worst eight hour exposure to range from 1.0-10 ppb. An additional 31,000,000 people may be exposed on the average to 1.1-2.0 ppb with possible 8 hour excursions to between 10.1 and 20 ppb. Among urban exposures due to auto exhaust it was estimated that 68,337,000 people were exposed at the lower doses described above and 45,353,000 at the higher dose conditions. It must be stressed that the intent of this report was to estimate exposure but not to measure responses to the exposure.

The final report U.S. EPA, 1979) in the series was an attempt by the EPA Carcinogen Assessment Group (CAG) to establish a risk assessment for ambient exposures to benzene and the final report was issued in 1979. Since that was a report on carcinogenesis, there was no emphasis on toxicity of benzene and accordingly, a risk assessment on the pancytopenic effects of benzene should be done independent of this CAG report. The CAG selected three studies on which to base their estimations. They were the studies by Infante, *et al*. (1977), Ott, *et al*. (1978) and Aksoy, *et al*. (1976, 1974), which reported on benzene-induced leukemia in rubber pliofilm plants in Ohio, Dow Chemical plants and shoe-making establishments in Turkey, respectively. Taking advantage of the levels of exposure reported by these investigators the CAG applied a linear non-threshold model to estimate the leukemogenic risk to the low average exposure populations cited in the exposure assessment report. A slope, for the linear dose-response curve was estimated by mathematically combining the data from the three reports and it was estimated that among the general population, 90 cases of leukemia, with 95% confidence limits of 24-235, are on an annual basis due to exposure to benzene in the 1 ppb range. This would account for from 0.23%-1.62% of all leukemia deaths in this country.

An important problem with the CAG report is the criticism of the estimates of exposure to benzene. In the Dow study (Ott, *et al*., 1978) the estimates were in all likelihood, the most accurate. There is less confidence in the exposure levels reported by Infante, *et al*. (1977) and Aksoy, *et al*. (1976, 1974). The exposure levels in the pliofilm plant reported by Infante, et al. (1977) were challenged at the benzene hearings where it was suggested that they were in reality much higher than proposed in the report. They were, in fact, not based on a carefully collected series of sampling data since the exposures occurred a number of years ago and the study was done retrospectively. The Aksoy, et al. (1976, 1974) sampling data, although reported in their publications, was not sufficiently documented to allow for evaluation of its accuracy. Nevertheless, given the non-threshold linear model for extrapolation adopted by CAG, the exposures in the Infante, et al., and Aksoy, et al., studies might have been much higher, but might not have had much more impact on the results of the CAG study.

The problems of benzene in drinking water are difficult to assess because of the paucity of data of the effects of ingested benzene in either humans or animals. From the point of view of carcinogenic risk assessment, however, the recent report of Maltoni and Scarnato (1979) will probably predominate in this field for some time to come. These workers administered benzene by gavage to Sprague-Dawley rats at doses of either 250 or 50 mg/kg, 4-5 days per week for 52 weeks. The authors report that, although the animals did not demonstrate acute or subacute toxic effects of benzene, there was a significant incidence of Zimbal gland tumors as well as increases in leukemias, mammary carcinomas and some other tumor types. Thus, they claim that benzene is a "general carcinogen," i.e., the carcinogenic effects are not restricted to leukemias.

The Ambient Water Quality Criteria Document (1980) from EPA contains an extensive discussion of the methods used in achieving a criterion for water levels of benzene. It was assumed that the major source of exposure was through inspired air. An adult male living in an urban environment could expect that 80% of his total exposure to benzene was via ambient air. A breakdown of total exposure showed that drinking water might contain anywhere from 0.1 to 10 ug/L of benzene, food contained 250 ug/L and ambient air 50 ug/m^3. The total intake was estimated to be 1.128 mg/day of which 1.4% came from water, 17.7% from food and 80.9% from air. The slope of the dose-response curve for benzene-induced leukemia in the CAG report was 0.024074 lifetime leukemia risks per ppm exposure to benzene in air. Making use of the fact that 1 ppm equals 3.25 mg/m^3, the respiratory rate is about 24 m^3/day and about 50% of the inhaled benzene is absorbed, it can be calculated, that the average daily intake of benzene at 1 ppm is 39 mg. They have calculated, however, that the intake of benzene which will increase the lifetime risk of benzene-induced leukemia by a factor of one in 10^5 is equal to 0.0162 mg/day.

Using an alternative approach to estimating benzene exposure it was suggested that the total body exposure to benzene may be as high as 1.1 mg/day, with air exposure the dominating route in these calculations as well.

It is unlikely that it can be confirmed that any specific case of environmental leukemia can be directly related to ambient benzene, nor is it likely that an animal experiment can be devised to demonstrate leukemia at low dose levels of benzene. The limiting factor in the determination of expected risks due to exposure to benzene is then the model that one uses for risk assessment. Although models other than linear non-threshold and one hit models have been proposed (Food Safety Council, The Scientific Committee, 1978) they have not gained acceptance. This is because they have not been validated and because they appear to be less conservative than the approach taken by EPA. Therefore, it is appropriate for the purposes of this document to make use of similar procedures in establishing a risk assessment for benzene in drinking water.

Chapter X Quantification of Toxicological Effects (QTE)

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Adjusted Acceptable Daily Intake (AADI) for the chemical is determined. For ingestion data, this approach is illustrated as follows:

$$\text{Adjusted ADI} = \frac{(\text{NOAEL or MEL in mg/kg})\ (70\ \text{kg})}{(\text{Uncertainty factor})\ (2\ \text{liters/day})}$$

The 70 kg adult consuming 2 liters of water per day is used as the basis for the calculations. A "no-observed-adverse-effect-level" or a "minimal-effect-level" is determined from animal toxicity data or human effects data. This level is divided by an uncertainty factor because, for these numbers which are derived from animal studies, there is no universally acceptable quantitative method to extrapolate from animals to humans, and the possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human toxicity data, an uncertainty factor is used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxins. The guidelines set forth by the National Academy of Sciences (Drinking Water and Health, Vol. 1 1977) are used in establishing uncertainty factors. These guidelines are as follows: an uncertainty factor of 10 is used if there exist valid experimental results on ingestion by humans, an uncertainty factor of 100 is used if there exist valid results on long-term feeding studies on experimental animals, and an uncertainty factor of 1000 is used if only limited data are available.

In the quantification of carcinogenic effects, mathematical models are used to calculate the estimated excess cancer risks associated with the consumption of a chemical through the drinking water. EPA's Carcinogen Assessment Group has used the linear non-threshold model, which is linear and does not exhibit a threshold, to extrapolate from high dose animal studies to low doses of the chemicals expected in the environment. This model estimates the upper bound (95% confidence limit) of the incremental excess cancer rate that would be projected at a specific exposure level for a 70 kg adult, consuming 2 liters of water per day, over a 70 year lifespan. Excess cancer risk rates also can be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. Current understanding of the biological mechanisms involved in cancer do not allow for choosing among the models. The estimates of incremental risks associated with exposure to low doses of potential carcinogens can differ by several orders of magnitude when these models are applied. The linear, non-threshold multi-stage model often gives one of the highest risk estimates per dose and thus would usually be the one most consistent with a regulatory philosophy which would avoid underestimating potential risk.

The scientific data base, which is used to support the estimating of risk rate levels as well as other scientific endeavors, has an inherent uncertainty. In addition, in many areas, there exists only limited knowledge concerning the health effects of contaminants at levels found in drinking water. Thus, the dose-response data gathered at high levels of exposure are used for extrapolation to estimate responses at levels of exposure nearer to the range in which a standard might be set. In most cases, data exist only for animals; thus, uncertainty exists when the data are extrapolated to humans. When estimating risk rate levels, several other areas of uncertainty exist such as the effect of age, sex, species and target organ of the test animals used in the experiment, as well as the exposure mode and dosing rates. Additional uncertainty exists when there is exposure to more than one contaminant due to the lack of information about possible additive, synergistic or antagonistic interactions.

Non-Carcinogenic Effects

The toxic effects of benzene in human and other animals include central nervous system effects, hematological effects as well as immune system effects. Short-term exposure to relatively high levels of benzene produces central nervous system effects that include dizziness, giddiness, exhilaration, nausea, vomiting, headache, drowsiness, staggering, loss of balance, narcosis, coma and death. It has been known since the 19th century that long term exposure to benzene produces adverse hematological effects; Santesson (1897) described cases of aplastic anemia in workers fabricating bicycle tires. The original association of acute leukemia with benzene exposure was made in 1928 (Delore and Bergomano, 1928) and it has been postulated that benzene may be a cause of acute myelogenous leukemia (Goldstein, 1981; OSHA, 1978b; NAS, 1980). Other hematological diseases also have been reported to be associated with benzene exposure (Goldstein, 1977).

Gerarde (1960) provides a table summarizing acute effects in which it is stated that 19,000-20,000 ppm for 5 to 10 minutes is a fatal benzene level; "7,500 ppm for 30 minutes is dangerous to life; 1,500 ppm for 60 minutes produces serious symptoms of illness; 50 to 150 ppm for five hours produces headache, lassitude and weakness". Mild central nervous system effects appear rapidly reversible following cessation of exposure. There is no evidence that they result in chronic brain damage. Also of importance is that these results appear to be concentration-dependent. Lower levels do not appear to illicit these responses no matter how long the exposure (at 480 mg/m^3)(Goldstein, 1977).

The toxicity of benzene to the hematopoietic system of humans experiencing chronic exposure to benzene is well documented. Reported effects include myelocytic anemia, thrombocytopenia (occurring separately or in cases of pancytopenia) and leukemia. In many of these studies, humans were exposed to benzene along with other solvents at relatively high concentrations. Data on the level and duration of exposure are inadequate for deriving dose-response relationships of chronic benzene toxicity (Vigliani and Formi, 1976). While it is impossible to determine a no-effect-dose, it is highly probable that continuous exposure to benzene at low levels (see below) will result in the above noted effects. Infante, et al. (1977), reported a retrospective cohort study of two populations of workers who were involved in the production of rubber sheeting (Pliofilm). In both plants during 1940-1949, the occupational exposure of 561 workers to benzene was apparently well within the maximum allowable concentration of 100 ppm that was usually recommended. Vital status to 1975, which was obtained for 75 percent of the workers, showed a significant excess of leukemia in those exposed to benzene, indicating a 10-fold increase in risk of death from myeloid and mononcytic leukemia.

In 1981, Rinsky, et al., published a study that was a follow-up using the same cohort as the Infante study. Rinsky's study followed up more than 98 percent of the vital status versus 75 percent for Infante's. Rinsky found that the leukemia mortality for those workers exposed five or more years had an Standard Mortality Ratio of 2,100. All leukemia deaths were either myelocytic or monocytic cell types.

The onset of leukemia is usually preceded by many observable effects on the hematopoietic system (Snyder and Kocsis, 1975). It is not known whether benzene causes leukemia as one aspect of its hematotoxic effects, whether the leukemia is a consequence of benzene-induced damage to immunological components of the bone marrow, or whether the leukemic effects are unrelated to the other hematopoietic manifestations (Laskin and Goldstein, 1977).

Benzene mixed with equal parts of olive oil was administered to rats by subcutaneous injection (Latta and Davies, 1941; Gerarde, 1956). Weight loss and leukopenia resulted from doses of 880 mg benzene/kg body weight, which were given daily for 14 days (Gerarde, 1956), and from doses of 1.32 g benzene/kg body weight, which were given daily for 3 to 60 days (Latta and Davies, 1941). In Latta and Davies' study, a rat that died after 10 days had hyperplastic bone marrow, and one that died at 21 days had acute leucopenia and hypoplastic bone marrow. Oral administration of benzene to rats in daily doses of 1, 10, 50 or 100 mg/kg weight during 132 days over

6 months resulted in leucopenia and erythrocytopenia at the lowest minimal effect level of 10 mg/kg and above (Wolf, et al., 1956).

Leucopenia is the most commonly observed effect of chronic benzene intoxication in laboratory animals. Deichmann, et al. (1963) exposed 40 male and 40 female Sprague-Dawley rats by inhalation to six different levels of benzene for 5 hours to 7 hours per day four days a week for six to 31 weeks. Tail blood was collected weekly or biweekly and analyzed for total peripheral white blood cell count, red blood cell count and benzene concentrations. All rats were examined for gross pathologic tissue changes and, in a few instances, the nucleated cell populations of femoral bone marrow were determined. The dose levels were 0, 50, 96, 146 or 2760 mg/m^3. The most significant and constant pathological changes were found in the lungs (chronic bronchopneumonia) and spleen (hemosiderosis). The splenic hemosiderosis was more severe and occurred more frequently in females when compared to controls, but was not dose related. Leucopenia developed at 146 mg/m^3 and above. This effect was dose related and occurred with greater severity and at an earlier time in females. In addition, there was some indication, also in females, that the circulating white blood cell count was depressed at 103 mg/m^3. However, at lower exposures, a fall in leukocytes causes cyclical fluctuations. Moreover, there is normally wide variation among cell counts during diurnal cycles and among individual animals.

Quantification of Non-carcinogenic Effects

OSHA noted that there was "no dispute" that certain non-maligant blood disorders in humans evidenced by a reduction in the level of red or white cells or platelets in the blood could result from exposures of 25 to 40 ppm (Pagnotio, et al., 1961, 43 Fed. Reg. at 5921). Several studies indicate that relatively slight changes in normal blood values could result from exposure below 25 ppm and perhaps below 10 ppm (Chang, 1972, Doskin, 1971).

Chang studied 119 workers exposed to benzene in an industrial area. Hematological abnormalities were observed in 28 percent including 21 with anemia, 2 with leukopenia, and 5 with both. Chang plotted a graph in which each of those affected individuals is characterized by duration of work on the abscissa and the level of benzene exposure on the ordinate. Based on this plot, Chang obtained an exponential function that implied a "threshold" of 10 ppm benzene for cytopenic effects (Chang, 1972). However, no hematological toxicity was observed in the 18 workers exposed to 10 to 20 ppm benzene. A major problem in interpreting this study is the absence of information concerning the definition of work exposure concentration for the individual employees.

Doskin (1971) in the Soviet Union evaluated 365 individuals employed for three years in what was apparently a new chemical factory. Serial hematological studies were performed on the exposed workers as well as the control group. Benzene exposure levels are given in terms of the maximum permissible concentration two- to eight-fold excess in 64 percent of the measurements in the first year (37% in the second year and 3% in the third year). This decrease in benzene levels paralleled a decrease in the number of workers who had hematological abnormalities. In the first year close to 40 percent of the workers exhibited mild hematological abnormalities, the most common being thrombocytopenia (95-155,000/mm^3). In as much as the maximal permissible concentration in the Soviet Union at the time of the study was apparently 5 ppm, these findings suggest that exposure of workers to concentrations of 10 to 40 ppm benzene for less than one year produces mild cytopenic effects. Interpretation of this study would be furthered by information concerning the benzene monitoring system and the actual levels recorded. It is hard to identify a high risk group per se. Since benzene bioaccumulates in the bone marrow, it would be surmised that those people with rapidly synthesizing marrows are at greatest risk. Those groups would include:

1. Fetuses, infants and children
2. People with anemia (women)
3. People with agranolocytemia (drug or chemically induced)

It is almost certain that nearly all environmental benzene exposure is a multiple chemical exposure. Gasoline, fuel oil and leachate being the most common mixtures. No one has determined if there is an additive or synergism of the effects of multiple chemical exposure. Certainly the acute effects will have combined greater effects if other volatile organic chemicals are present.

An Average Daily Intake (ADI) may be calculated using data from Wolfe, et al. (1976) who gavaged female rats of 1, 10, 50 or 100 mg/kg over a 187 day period. No effects were seen at 1 mg/kg but a slight leucopenia was observed at 10 mg/kg given 132 times over the 187 days. The ADI may be calculated using the 1 mg/kg level as a no-observed-adverse-effect-level (NOAEL) and assuming 100 percent absorption factor.

$$\text{ADI} = \frac{1\ \text{mg/kg} \times (100\%)(5)}{(100)(10)\qquad(7)} \times 70 = 0.50\ \text{mg/day} \times 10^{-4}\ \text{kg/day} =$$

$$50\ \text{ug}/70\ \text{kg/day}$$

Where: 1 mg/kg = assumed NOAEL

70 kg = 70 kg man

100% = absorption

100 = uncertainty factor appropriate for use with NOAEL from animal data and no equivalent human data

10 = uncertainty factor for less than lifetime exposure

An ADI based upon a NOAEL for human hematological abnormalities might be developed as follows:

The NOAEL is between 10 ppm and 25 ppm.

$$\frac{10\ \text{ppm} \times 3.2\ \text{mg/m}^3/\text{ppm} \times 8\ \text{m}^3 \times 0.6}{100 \times 10} = 0.1530\ \text{mg/day}$$

Where: 1 ppm = $3.2\ \text{mg/m}^3$

$8\ \text{m}^3$ = The amount of air a 70 kg worker breaths in 8 hours/ per day

0.6 = 60% = percent absorbed and retained

100 = uncertainty factor appropriate for human exposure with less than ideal experimental conditions

10 = uncertainty factor for less than a lifetime exposure

Adjusted ADI would be derived thusly:

$$\frac{(1\ \text{mg/kg})(70\ \text{kg})(100\%)(5)}{(100)\ \text{X}\ \ (10)\ \text{X}\ 2\ \text{l}\ \text{X}\ (7)} = 0.025\ \text{mg/l or}$$

$$\frac{\text{ADI}}{2\ \text{l}}$$

Where: 1 mg/kg = assumed no-observed-adverse-effect-level (NOAEL)

70 kg = 70 kilogram man

100% = absorption factor

2 l = 2 liter of water consumed per day by the 70 kg adult

100 = uncertainty factor appropriate for use with NOAEL from animal data and no equivalent human data

10 = factor for less than lifetime exposure

5/7 = factor to correct from 5 days/week to 7 days

An Adjusted ADI for non-carcinogenic effects based upon a NOAEL for human hematological abnormalities might be developed as follows:

The NOAEL is between 10 ppm and 25 ppm.

$$\text{Adjusted ADI} = \frac{10\ \text{ppm X } 3.2\ \text{mg/m}^3\text{/ppm X } 8\ \text{m}^3\text{/day X } 0.6}{100\ \text{X}\ 10} = 0.078\ \text{mg/day}$$

$$\text{or } \frac{0.1536\ \text{mg/l}}{2} = 0.078\ \text{mg/l}$$

Where: 1 ppm = 3.2 mg/m^3

$8\ m^3$ = The amount of air a 70 kg person breaths per active work day

0.6 = 60% = percent absorbed and retained

100 = uncertainty factor appropriate for human exposure with less than ideal experimental conditions

10 = uncertainty factor for less than a lifetime exposure

Where: 21 = volume of drinking water imbbed a day by a 70 kg adult

From the series of longer term experiments described above, one would develop an Adjusted ADI protective against non-carcinogenic effects in the range of 0.025 to 0.078 mg/day for the 70 kg adult based on the human data. This assumes that drinking water is the sole source of exposure to benzene.

The Adjusted ADI is derived to reflect allowable daily exposure of a 70 kg adult drinking two liters of water per day, and whose sole source of exposure to benzene is via that drinking water. This calculation does not reflect the associated carcinogenic risk.

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Carcinogenic Effects

Maltoni and Scarnato (1979) administered by gavage benzene dissolved in virgin olive oil to 13 week old Sprague-Dawley rats. The material was administered at doses of 50 or 250 mg/kg for 4 to 5 days a week for 52 weeks. The animals then were allowed to live until spontaneous death. Each high dose group consisted of 35 male and 35 female rats; the controls and low dose groups were composed of 30 male and 30 female rats each. After 20 weeks of exposure, Maltoni and Scarnato corrected the denominators (numbers of animals surviving) to reflect "nonexperimentally" caused deaths. The 250 mg/kg dose level group then consisted of 33 male and 32 female rats; the 50 mg/kg and olive oil control group then consisted of 28 male and 30 female rats. The authors reported their results after 144 weeks. At the 250 mg/kg dose level, 25 percent (8/32) of the female rats had Zymbal gland tumors, 6.2 percent (2/32) had skin carcinomas, 21.9 percent (7/32) had mammary carcinomas, 3.1 percent (1/32) had leukemias. The male rats in the 250 mg/kg dose group had no Zymbal gland tumors, no skin carcinomas and no mammary gland tumors; however, they had 12.1 percent leukemias (4/33), one subcutaneous anigosarcoma (3.0%) and one hematoma (3.0%). In the rats remaining after the 20 week adjustments, the following carcinogenic effects were noted. At the 50 mg/kg dose level, only female rats had tumors which were Zymbal gland carcinoma, 6.7 percent (2/25) and mammary carcinoma, 13.3 percent (4/25). The control group had tumors only in female rats, which were mammary carcinoma 10.0 percent (3/30), and leukemias, 3.3 percent (1/30). The authors concluded that benzene "appears to cause Zymbal gland carcinomas, at the two studied dose levels with a dose response relationship. Moreover, a dose correlated increase of hemato-lympho reticular neoplasias (leukemias) and mammary carcinomas has also been observed".

Ward, et al. (1975) subcutaneously injected male C57BL/6N mice repeatedly with benzene dissolved in corn oil. Eighty benzene treated mice, while initially divided into four groups ranging from 0.1 to 2.0 mg/kg, were eventually combined and reported as a single experimental group. Three control groups were used with twenty male mice per group: a no treatment control, a corn oil only control, and a positive control using butylnitrosourea. The animals were injected twice weekly for 44 weeks, then once weekly until 54 weeks. At 104 weeks after the first injection, all surviving mice were sacrified (108 weeks of age) and a complete necropsy was performed, as had been done with all the mice that died. The toxic lesions included bone marrow depleted of hematopoietic cells and hepatonecrosis. A granulocytic leukemia was also noted.

After reviewing the data from that study, the National Academy of Sciences Safe Drinking Water Committee concluded that the increase in pathology was not statistically significant, even when time to response was incorporated into the analysis (National Academy of Sciences, 1977).

Benzene has been shown to be carcinogenic in Sprague-Dawley rats. As noted above, tumors were found at both 50 mg/kg and 250 mg/kg dose levels. It has been shown that humans are at least two orders of magnitude more sensitive than animals.

Over the past several decades, scientists have conducted a great deal of research in an effort to establish the mechanism(s) by which chemical substances exert their carcinogenicity. The somatic cell mutation theory of carcinogenicity suggests that for a carcinogenic response to occur, an irreversible change must occur in the cell which results in proliferation of a neoplasm. This change reflects a mutational event in the DNA of that cell, suggesting that the chemical carcinogen must interact directly with or otherwise alter the DNA to initiate the change. In recent years, however, some substances have been shown to be carcinogenic, but by mechanisms in which there apparently is no direct interaction with or alteration of the DNA of the cell by the substance. Presumably, these compounds are not capable of initiating the alteration of a normal cell to a neoplastic response in latent cells. On the basis of these purported differences in mechanisms, carcinogens now are often classified into two broad categories: genotoxic and epigenetic or nongenotoxic.

The mechanisms by which a compound exerts its carcinogenicity rarely can be determined by the chronic testing of whole animals such as is done in the NTP bioassay. Thus, a Large number of short-term *in vitro* and *in vivo* assay systems have been developed for the purpose of elucidating mechanisms. Since most of the *in vitro* testing systems measure mutational events, and many carcinogens are mutagens, it is suggested that positive results in certain of these test systems may indicate genotoxicity. The decision as to whether a substance is genotoxic and be made qualitatively on the basis of several criteria: 1) a reliable, positive demonstration of genotoxicity in appropriate prokaryotic and eukaryotic systems *in vitro*; 2) studies on binding to DNA and 3) evidence of biochemical or biologic consequences of DNA damage (Weisburger and Williams, 1981).

No single test system appears capable of detecting all carcinogens that are genotoxic. Therefore, a number of scientists have proposed testing batteries such that results from each test within the battery, when evaluated as a whole, will allow one to make a conclusion about the mechanism of carcinogenicity of a particular compound.

Benzene has not been systematically studied in any specific battery of tests, but has been evaluated in a number of test systems that have been proposed for inclusion in one or more batteries.

Toxic effects on bone marrow cells of rats and other

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laboratory animals include changes in chromosome number and chromosome breakage that resemble those in humans. There is no clear evidence for dose-dependent response (Laskin and Goldstein, 1977). Lyon (1975) used the Ames assay with Salmonella typhimurium strains TA98 and TA100 to test benzene for mutagenicity in doses ranging from 0.1 to 1.0 ul/plate, both without and with microsomal fraction at concentrations from 1 to 50 ul/plate. Postmitochondrial supernatant suspensions of mircosomes were prepared from liver homogenates from normal rats and from rats that had been treated with phenobarbital and 3-methylcholanthrene (MCA), and from the bone marrow of normal and MCA-treated rats. Benzene was uniformly negative in all of these assays and was inactive in the dominant lethal assay in rats.

When considering the weight of evidence as a whole, it becomes evident that benzene may exert its carcinogenic effect via non-genotoxic mechanisms. Since all living mammalian organisms have probably initiated cellular system from the earliest time in life, one must now try to estimate the risk to humans that exposure to this substance in drinking water would pose. It is well known that both CAG (U.S. EPA, 1980) and OSHA (1978b) have determined benzene to be a human carcinogen. Maltoni and Scartano (1979, 1980) have demonstrated benzene to be an animal carcinogen. Benzene appears to have a unique mechanism for producing cancer. Scientists have not been able to demonstrate a threshold for benzene. Therefore, exposure to any amount of this type of toxicant obligates some measure of risk. Since we do not have experimental results for effects seen at low doses of exposure, we may mathematically extrapolate from the results obtained at higher doses to project what would expected at the lower doses.

Quantification of Carcinogenic Effects

Using methodology described in detail elsewhere, both the National Academy of Sciences and the EPA's Carcinogen Assessment Group(CAG) have calculated estimated incremental excess cancer risks associated with the consumption of benzene. Each group used the linearized, non-threshold multistage model.

The National Academy of Sciences, in Drinking Water and Health, Volume 3 (1980), states:

> There are no data from animal models for use in extrapolation. Occupational studies on human exposure (Aksoy, et al., 1972, 1974a, b, 1976; Ishimaru, et al., 1971: Thorpe, 1974) do not contain adequate information on degree of exposure or size of population at risk. In addition, the workers in benzene-related occupations typically were exposed to other chemicals, as in the study reported by Ott, et al., 1978. Consequently, extrapolation of benzene-induced cancer risk from such data as these would be tenuous.
>
> In a study by Infante, et al., 1977, workers were exposed to benzene as the sole chemical suspected of affecting the hematopoietic system. In these cases, benzene concentrations apparently were high during the first years of exposure and were lower thereafter. There are no data indicating how often short exposures at elevated levels may have occurred. Estimates of actual exposure are inadequate for extrapolation for risk of benzene-induced leukemia.

The EPA's Carcinogen Assessment Group (CAG) has determined a carcinogenic risk estimate by using the following considerations (see U.S. EPA Ambient Water Quality Documents Benzene):

> Three epidemiology studies of workers exposed to benzene vapors on their jobs, performed by Infante, Ott and Aksoy, were reviewed by the CAG for the Office of Air Quality Planning and Standards (U.S. EPA, 1979). Their result was that the potency for humans breathing benzene continuously is B = 0.02407. This means that the lifetime risk of getting leukemia, R equals 0.02407 times the lifetime average continuous exposure, X, measured as ppm of benzene by volume in air, or R = B X. Therefore, the air concentration, X, resulting in a risk of 10^{-5} is $X = R/B = 10^{-5}/0.02407 = 4.1539 \times 10^{-4}$ ppm.
>
> Since the air concentration corresponding to 1 ppm of benzene is 3.25 mg/m^3 and assuming a respiratory rate of 20 m^3/day and a respiratory rate of 20 m^3/day and a respiratory absorption coefficient of 0.50, the daily intake that would result in a risk of 10^{-5} is:

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$$4.154 \times 10^{-4} \text{ ppm} \times 3.25 \times 10^{3} \text{ ug/m}^{3} \text{ per ppm} \times 20\text{m}^{3}\text{/day} \times 0.5 = 13.5 \text{ ug/day}$$

If it is assumed that the fraction of benzene absorbed is the same between inhalation and ingestion of water and fish, a daily benzene intake of 13.5 ug through drinking water would cause a leukemia risk of 10^{-5}. The water concentration given this intake is:

$$C = (13.5 \text{ ug/day})/(2)$$
$$= 6.75 \text{ ug/l}$$
$$= 6.8 \text{ ug/l}$$

Table 1

Exposure Assumptions (per day)	Excess Lifetime Cancer Risk	CAG (upper 95% CL) Corresponding Criteria
2 liters of drinking water	10^{-6}	0.68
	10^{-5}	6.8
	10^{-4}	68.0

IARC reviewed Benzene in Vol. 29 and stated that benzene is a human carcinogen. The IARC working group using the Rinskey data developed minimum estimates of 140-170 excess leukemia deaths per 1000 exposed workers over a working lifetime. This calculation was based upon a 100 ppm exposure level in air. They declined to give an upper bound figure for the 95% confidence limit for excess leukemias following exposures over a working lifetime, i.e. from 20 years to end of life, taken at age 75.

XI. REFERENCES

Abou-el-Makarem, M.M., P. Millburn, R.L. Smith, R.R. Williams. 1967. Biliary excretion of foreign compounds: Benzene and its derivatives in the rat. Biochem. J. 105:1269.

Abramova, Z.I., I.D. Gadaskina. 1965. Inhibition of benzene oxidation by certain antoxidants. Fed. Proc. Transl. Suppl. 1966. 25:T-91-92. Originally appeared in: Gigiena Trude i Professional'nye Zabolevaniya.

Aksoy, M. 1976. Types of leukemia in chronic benzene poisoning, A study in 34 patients. Acta Haemat. 55:65-72.

Aksoy, M. 1977. Testimony before Occup. Safety Health Admin. U.S. Dept. Labor. July, 1977.

Aksoy, M., S. Erdem. 1969. Some problems of hemoglobin patterns in different thalassemic syndromes showing the heterogeneity of betathalassemic genes. Ann. of N.Y. Acad. Sci. 165:13.

Aksoy, M., S. Erdem. 1978. Follow-up study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic exposure to benzene. Blood. 52:285.

Aksoy, M., S. Erdem, T. Akgun, O. Okur, K. Dincol. 1966. Osmotic fragility studies in three patients with aplastic anemia due to chronic benzene poisoning. Blut. 13:85.

Aksoy, M., K. Dincol. T. Akgun, S. Erdem, G. Dincol. 1971. Haematological effects of chronic benzene poisoning in 217 workers. Br. Jour. Ind. Med. 28:296.

Aksoy, M., K. Dincol, K. Erdem, T. Akgun, G. Dincol. 1972a. Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Brit. Jour. Industr. Med. 29:56.

Aksoy, M., K. Dincol, S. Erdem, G. Dincol. 1972b. Acute leukemia due to chronic exposure to benzene. Am. Jour. Med. 52:160.

Aksoy, M., S. Erdem, G. Erdogan, G. Dincol. 1974a. Acute leukemia in two generations following chronic exposure to benzene. Hum. Hered. 24:70.

Aksoy, M., S. Erdem, G. Dincol. 1974b. Leukemia in shoe workers exposed chronically to benzene. Blood. 44:837.

Aksoy, M., S. Erdem, K. Dincol, T. Hepyuksel, G. Dincol. 1974c. Chronic exposure to benzene as a possible contributory etiological factor in Hodgkin's disease. Blut. 38:293.

Aksoy, M., et al., 1975a. The reaction of normal and thalassaemic individuals to benzene poisoning: The diagnostic significance of such studies. In: Abnormal haemoglobins and thalassaemia. Diagnostic Aspects. Academic Press, Inc., NY.

Aksoy, M., S. Erdem. G. Dincol. 1975b. Two rare complications of chronic benzene poisoning: myeloid metaplasia and paroxysmal nocturnal hemoglobinuria. Report of two cases. Blut. 30:255.

Aksoy, M., S. Erdem, G. Erdogan, G. Dincol. 1976a. Combination of genetic factors and chronic exposure to benzene in the aetiology of leukemia. Hum. Hered. 26:149.

Amiel, J.L. 1960. Essai negatif d'induction de Leucemies chez les souris par le benzene. Rev. franc. etudes clin, et biol. 5:198-199.

Andjelkovic, D., J. Taulbee, M. Symons. 1976. Mortality experience of a cohort of rubber workers, 1964-1973. Jour. Occup. Med. 18:387.

Andjelkovic, D., J. Taulbee, M. Symons, T. Williams. 1977. Mortality of rubber workers with reference to work experience. Jour. Occup. Med. 19:397.

Andrews, L.S., E.W. Lee, C.M. Witmer, J.J. Kocsis, R. Snyder. 1977a. Effects of toluene on the metabolism, disposition and hemopoietic toxicity of ^{3}H-benzene. Biochem. Pharmacol. 26:293-300.

Andrews, L.S., H.A. Sasame, J.R. Gillette. 1979. ^{3}H-Benzene metabolism in rabbit bone marrow. Life Sci. 25:567-572.

Andrews, L.S., B.R. Sonawane, S.J. Yaffe. 1977b. Characterization and induction of aryl hydrocarbon benzo(a)pyrene hydroxylase in rabbit bone marrow. Res. Commun. Chem. Patho. Pharmacol. 15:319-320.

Angeline, P., C. Merritt, Jr. 1975. Effect of irradiation on volatile constituents of stored haddock flesh. Jour. Food Sci. 40:197-199.

Anonymous. 1973. Busy gasoline pumps put benzene users in a bind. Chemical Week. p. 11. August 8.

Ayers, G.W., R.E. Muder. 1964. Benzene. In: Stanton A., ed. Kirk-Othmer encyclopedia of chemical technology, 2nd ed. John Wiley and Sons, Inc., New York, vol. 3, pp. 367-401.

Bergman, K. 1979. Whole body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents. Scand. J. Work Env. Hlth. 5(1):64.

Boje, V.H., W. Beukel, H.J. Heiniger. 1970. Untersuchungen zur leukopoese im Knochenmark der ratte nach chronisches benzol-inhalation. Blut. 21:250-257.

Buttery, R.G., R.M. Seifert, L.C. Ling. 1975. Characterization of some volatile constituents of dry red beans. Jour. Agr. Food Chem. 23:516-519.

Camp, W.A., E.A. Baumgartner. 1915. Inflammatory reactions in rabbits with a severe leukopenia. J. Exptl. Med. 22:174-192.

Cesaro, A.N. 1946. Is percutaneous absorption of benzene possible? Med. Lavozo 4:151-156.

Chang, I.W. 1972. Study on the threshold limit value of benzene and early diagnosis of benzene poisoning. Jour. Cath. Med. Coll. 23:429.

Conca, G.L., A. Maltagliati. 1955. Study of the percutaneous absorption of benzene. Med. Lavoro 46:194-198.

Cornish, H.H., R.C. Ryan. 1965. Metabolism of benzene in non-fasted, fasted and aryl-hydroxylase inhibited rats. Toxicol. Appl. Pharmacol. 7:767-771.

Day, E.A., D.F. Anderson. 1965. Gas chromatographic and mass spectral identification of natural components of the aroma fraction of blue cheese. Jour. Agr. Food Chem. 13:2-4.

Day, E.A., L.M. Libbey. 1964. Cheddar cheese flavor: gas chromatographic and mass spectral analyses of the neutral components of the aroma fraction. Jour. Food Sci. 29:583-589.

Dean, B.J. 1978. Genetic toxicology of benzene, toluene, xylenes and phenols. Mut. Res. 47:75.

DeGowin, R.L. 1963. Benzene exposure and aplastic anemia followed by leukemia 15 years later. Jour. Am. Med. Assoc. 185:748.

Deichmann, W.B., W.E. MacDonald, E. Bernal. 1963. The hemopoietic toxicity of benzene vapors. Toxicol. Appl. Pharmacol. 5:210-224.

Delore, P., C. Borgomano. 1928. Leucemie aigue an cours de l'intoxication benzenique. Sue l'origine toxique de certaines leucemies aigues et leurs relations avec les anemies graves. Jour. Med. Lyon. 9:227.

Diem, K., ed. 1962. Documenta geigy, scientific tables. 6th edition, p. 274.

Diwan, B.A., H. Meier. 1976. Carcinogenic effects of a single dose of diethylnitrosamine in three unrelated strains of mice: Genetic dependence of the induced tumor types and incidence. Can. Lett. 1:249.

Doskin, T.A. 1971. Effect of age on the reaction to a combination of hydrocarbons. Hygiene and Sanitation. 36:379.

Dowty, B.J., D.R. Carlisle, J.L. Laseter. 1975. New Orleans drinking water sources tested by gas chromatography-mass spectrometry: Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environ. Sci. Technol. 9:762-765.

Drew, R.T., J.R. Fouts. 1974. The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol. Appl. Pharmacol. 27:183-193.

Drew, R.T., J.R. Fouts, C. Harper. 1974. The influence of certain drugs on the metabolism and toxicity of benzene in Symposium on Toxicology of Benzene and Alkylbenzenes (Braun, D., ed.), p. 17-31, Industrial Health Foundation, Pittsburgh.

D'Souza, M., R. Snyder, J.J. Kocsis. 1979. Benzene inhibits ovarian hypertrophy in the hemispayed rat. Abstracts of Papers, 18th Annual Meeting, Society of Toxicology, A40.

Fentiman, A.F., M.B. Neher, G.W. Kinzer, P.R. Sticksel, R.M. Coatant, G.A. Jungclaus. 1979. Environmental monitoring benzene. Battelle Columbus Laboratories, Columbus, OH. PB-295 641.

Fick, J.E. 1976. To 1985: U.S. benzene supply/demand. Hydrocarbon Processing. 55:127-129.

Flath, R.A. and R.R. Forrey. 1970. Volatile components of smooth cayenne pineapple. Jour. Agric. Food Chem. 18:306-309.

Food Safety Council, the Scientific Committee. 1978. Proposed system for food safety assessment. Food and Cosmetics Toxicology. 16:109-136.

Forni, A., A. Cappellini, E. Pacifico, E. Vigliani. 1971b. Chromosome changes and their evolution in subjects with past exposure to benzene. Arch. Environ. Health. 23:385.

Forni, A., L. Moreo. 1969. Chromosome studies in a case of benzene-induced erytholeukemia. Europ. Cancer. 5:459.

Forni, A., L. Moreo. 1967. Cytogenetic studies in a case of benzene-induced leukemia. Europ. J. Cancer. 3:251-255.

Forni, A., E. Pacifico, A. Limonta. 1971a. Chromosome studies in workers exposed to benzene or toluene or both. Arch. Environ. Health. 22:373-354.

Forni, A., E.C. Vigliani. 1974. Chemical leukemogenesis in man. Ser. Haemat. 7:210.

Funes-Cravioto, F., B. Kolmodin-Hedman, J. Lindsten, M. Nordenskjold, C. Zapata-Gayon, B. Lambert, E. Norberg, R. Olin. 1977. Chromosome aberrations and sister-chromatid exchange in workers in chemical factories and in children of women laboratory workers. The Lancet. Aug. 13, pp. 322-325.

Gerarde, H.W. 1960. Toxicology and biochemistry of aromatic hydrocarbons. Elsevier Publishing Co., NY.

Girard, R., L. Revol. 1970. La frequence d'une exposition benzenique au cours hemopathies graves. Nouv Revue Fr. Hemat. 10:477.

Gofmekler, V.A. 1968. Effect on embryonic development of benzene and formaldehyde in inhalation experiments. Hyg. Sanit. 33:327-332.

Goldstein, B.D. 1977. Hematotoxicity in humans. Benzene toxicity, a critical evaluation. S. Laskin and B. Golstein (eds.). Toxicol. Environ. Health Suppl. 2.

Goldwater, L.J. 1941. Disturbances in the blood following exposure to benzene (benzol). Jour. Lab. Clin. Med. 26:957.

Goldwater, L.J., M.P. Tewksbury. 1941. Recovery following exposure to benzene (benzol). Jour. Indust. Hyg. 23:217.

Gonasun, L.M., C.M. Witmer, J.J. Kocsis, R. Snyder. 1973. Benzene metabolism in mouse liver microsomes. Toxicol. Appl. Pharmacol. 26:398-406.

Goth, R., M.F. Rajewsky. 1974. Persistence of O^6-ethylguanine in rat brain DNA: Correlation with nervous system-specific carcinogenesis by ethylnitrosourea. Proc. Natl. Acad. Sci., USA 71:639.

Green, J.D., B.K.J. Leong, S. Laskin. 1978. Inhaled benzene fetotoxicity in rats. Toxicol. Appl. Pharmacol. 46:9-18.

Green, J.D., C.A. Snyder, J. LoBue, B.D. Goldstein, R.E. Albert. 1981. Acute and chronic dose/response effects of inhaled benzene on multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-1 mice. Toxicol. Appl. Pharmacol. 58:492-503.

Greenburg, L., M.R. Mayers, L. Goldwater, A.R. Smith. 1939. Benzene poisoning in rotogravure printing. Jour. Industr. Hyg. Toxicol. 21:395.

Greene, M.H., R.N. Hoover, R.L. Eck, J.F. Fraumeni, Jr. 1979. Cancer mortality among printing plant workers. Environ. Res. 20:66.

Greenlee, W.F., E.A. Gross, R.D. Irons. 1980. A study of the disposition of ^{14}C-labeled benzene metabolites using whole body autoradiography. Abstracts of Papers, Society of Toxicology, 19th Annual Meeting, A52.

Guasch, J., *et al*., 1959. Anemia benzolica complicada con leucemia aguda a los seis anos de evoluction. Sangre. 4:129.

Guberan, E., P. Kocher. 1971. Pronostic lointain de l'intoxication benzolique chronique: controle d'une population 10 ans apres l'exposition. Schweiz. Med. Wochenschr. 101:1789.

Gut, I. 1978. Some factors affecting xenobiotic metabolism *in vivo*, in Industrial and Environmental Xenobiotics. *In vitro* versus *in vivo* biotransformation and toxicity, Fouts, J.R. and I. Gut (eds.) pp. 74-82, Excerpta Medica, Amsterdam.

Hamilton, A. 1922. The growing menace of benzene (benzol) poisoning in American industry. J. Amer. Med. Assoc. 78:627.

Hamilton, A. 1928. The lessening menace of benzol poisoning in American industry. J. Ind. Hyg. 10:227.

Hamilton, A. 1931. General review. Benzene (benzol) poisoning. Arch. Path. 11:434.

Hammond, J.W., E.R. Herman. 1960. Industrial hygiene features of a petrochemical benzene plant design and operation. Amer. Ind. Hyg. Assoc. J. 21:173-177.

Harigaya, K., M.E. Miller, E.P. Cronkite, R.T. Drew. 1981. The detection of *in vivo* hematotoxicity of benzene by *in vitro* liquid bone marrow cell cultures. Toxicol. Appl. Pharmacol. 60:346-353.

Harper, C., R.T. Drew, J.R. Fouts. 1975. Species differences in benzene hydroxilation to phenol by pulmonary and hepatic microsomes. Drug Metabol. and Disposition 5:381-388.

Hartman, H.A., E.C. Miller, J.A. Miller, F.K. Morris. 1959. The leukemogenic action of 2-acetylaminophenanthrene in the rat. Can. Res. 19:210.

Hartwich, G., G. Schwanitz, J. Becker. 1969. Chromosome anomalies in a case of benzene leukemia. Germ. Med. Mth. 14:449-450.

Hektoen, L. 1916. The effects of benzene on the production of antibodies. J. Infec. Dis. 19:69-84.

Hernberg, S., M. Savilahti, K. Ahlman, A. Asp. 1966. Prognostic aspects of benzene poisoning. Brit. Jour. Industr. Med. 23:204.

Hirokawa, T., K. Nomiyama. 1962. Studies on the poisoning by benzene and its homologues. Oxidation rate of benzene in the rat liver homogenate. Med. J. Shinshu Univ. 7:29-39.

Hirschfelder, A.D., M.C. Winternitz. 1913. Studies upon experimental pneumonia in rabbits: Part IV. Is there a parallelism between trypanocidal and pneumococcidal action of drugs? J. Exptl. Med. 17:666-678.

Horsley, L.H. 1947. Table of azeotropes and nonazeotropes. Industrial and Engineering Chemistry, Analytical Chemistry. 19:508-600.

Hough, H., S. Freeman. 1944. Relative toxicity of commercial benzene and a mixture of benzene, toluene and xylene. Fed. Proc. 3:20.

Howard, P.H., P.R. Durkin. 1974. Sources of contamination, ambient levels, and fate of benzene in the environment. U.S. Environmental Protection Agency, Washington, D.C. EPA 560/575-005.

Hudak, A., G. Ungvar. 1978. Embryotoxic effects of benzene and its methyl derivatives: Toluene, xylene. Toxicology. 11:55-63.

Huggins, C.B., T. Sugiyama. 1966. Proc. Natl. Acad. Sci. 55:74.

Hunter, C.G. 1968. Solvents with reference to studies on the pharmacodynamics of benzene. Proc. R. Soc. Med. 61:913.

Hunter, D. 1962. The diseases of occupations. Little, Brown and Company, Boston p. 494. 497.

Ikeda, M., H. Ohtsuji. 1971. Phenobarbital-induced protection against toxicity of toluene and benzene in rats. Toxicol. Appl. Pharmacol. 20:30-43.

Ikeda, M. 1964. Enzymatic studies on benzene intoxication. J. Biochem. (Tokyo) 55:231-243.

Infante, P.F., J.K. Wagoner, R.A. Rinsky, R.J. Young. 1977a. Leukemia in benzene workers. The Lancet. 2:76-78.

Infante, P.F., R.A. Rinsky, J.K. Wagoner, R.J. Young. 1977b. Benzene and leukemia. Lancet. 2:867.

International Agency for Research on Cancer. 1974. Some antithyroid and related substances, nitrofurons and industrial chemicals. In: IARC monographs on the evaluation of the carcinogenic risk of chemicals to man. Volume 7. Lyon, France. pp. 203-221.

Irons, R.D., J.G. Dent, T.S. Baker, D.E. Rickert. 1980. Benzene is metabolized and covalently bound in bone marrow *in situ*, Chem. -Biol. Interactions 30:241-245.

Irons, R.D., W.F. Greenlee, D. Wierda, J.S. Bus. (in press). Relationship between benzene metabolism and toxicity: A proposed mechanism for the formation of reactive intermediates from polyphenol metabolites.

Ishimaru, T., H. Okada, T. Tomiyasu, T. Tsuchimoto, T. Hoshino, M. Ichimaru. 1971. Occupational factors in the epidemiology of leukemia in Hiroshima and Nagasaki. Am. Jour. Epidemiol. 93:157.

Jablonska, J.K., K. Knobloch, J. Majka, J.M. Wisniewska-Knypl. 1975. Stimulatory effects of chlordiazepoxide, diazepam, and oxazepam on the drugmetabolizing enzymes in microsomes. Toxicology 5:103-111.

Jenkins, L.J., R.A. Jones, J. Siegel. 1970. Long-term inhalation screening studies of benzene, toluene, O-xylene and cumene on experimental animals. Toxicol. Appl. Pharmacol. 16:818-823.

Jerina, D., J. Daly, B. Witkop, P. Zaltzman-Nirenberg, S. Udenfriend. 1968. Role of the arene oxide-oxpin system in the metabolism of aromatic substrates. 1. *In vitro* conversion of benzene oxide to a premercappuric acid and a didhydrodiol. Arch. of Biochem. and Biophys. 128:176-183.

Jerina, D., J.W. Daly. 1974. Arene oxides: A new aspect of drug metabolism, Science 185:573-582.

Justin-Besancon, L., *et al*. 1959. Leucoise aigue survenue 27 ans apres exposition aux vapeurs benzoliques. La Semaine des Hopitaux. 35:168.

Kahn, H.A., V.I. Muzyka. 1973. The chronic effect of benzene on prophyrin metabolism. Work Environ. Health. 10:140.

Kahn, H., M.H. Kahn. 1973. Cytogenetische Untersuchungen bei chronischer. Benzol-exposition. Arch. Toxikol. 31:39.

Kinlin, T.E., R. Muralidhara, A.O. Pittet, A. Sanderson, J.P. Walradt. 1972. Volatile components of roasted filberts. Jour. Agr. Food Chem. 20:1021-1028.

Kirschbaum, A., L.C. Strong. 1942. Influence of carcinogens on the age incidence of leukemia in the high leukemia F strain in mice. Can Res. 2:841-845.

Kissling, M., B. Speck. 1969. Chromosome aberrations in experimental benzene intoxication. Helv. Med. Acta 36:59.

Kissling, M., B. Speck. 1972. Further studies on experimental benzene induced aplastic anemia. Blut. 25:97-103.

Kocsis, J.J., S. Harkaway, M.C. Snatoyo, R. Snyder. 1968. Dimethyl sulfoxide: Interactions with aromatic hydrocarbons. Science 160:427-428.

Laerum, O.D. 1973. Reticulum cell neoplasms in normal and benzene treated hairless mice. Acta Path. Microbiol. Scand. 81:57-63.

Lange, A.L., G.M. Forker. 1961. Handbook of chemistry. McGraw-Hill, New York, pp. 34, 1484-1493.

Lange, A., R. Smolik, W. Zatonski, J. Syzmanska. 1973a. Serum immunoglubulin levels in workers exposed to benzene, toluene and xylene.

Latta, J.S., L.T. Davies. 1941. Effects on the blood and hemopoietic organs of the albino rat of repeated administration of benzene. Arch. Pathol. 31:55-67.

Lazarew, N.W., Brussillowskaja, J.N. Lawrow, F.B. Lifschitz. 1931. Cutaneous permeability for petroleum ether and benzene. Arch. f. Hyg. 106:112-122.

Lee, E.W., J.J. Kocsis, R. Snyder. 1974. Benzene: Acute effect on ^{59}Fe incorporation into circulating erythorcytes, Tox. Appl. Pharmacol. 27:431-436.

Lee, E.W., J.J. Kocsis, R. Snyder. 1973. Dose-dependent inhibition of ^{59}Fe incorporation into erthrocytes after a single dose of benzene. Res. Commun. Chem. Pathol. Pharmacol. 5:547.

Li, F.P., J.F. Fraumeni, Jr., N. Mantel, R.W. Miller. 1969. Cancer mortality among chemists. Jour. Natl. Cancer Instr. 43:1159.

Lignac, G.O.E. 1932. Die Benzolleukamie bei Menschen and weissen Mausen. Klin. Wschr. 12:109.

Litton Bionetics, Inc. 1977. Mutagenicity evaluation of benzene, final report. Submitted to the American Petroleum Institute, Washington, D.C. in March 1977. LBI Project No. 2697. Litton Bionetics, Inc., Kensington, M.D., p. 55.

Longacre, S.L., J.J. Kocsis, R. Snyder. 1980. Benzene metabolism and toxicity in CD-1, C57/B6 and DBA/2N mice, in Microsomes, Drug Oxidations and Chemical Carcinogenesis, Coon, M.J., A.H. Conney, R.W. Estabrook, H.V. Gelboin, J.R. Gillette and P.J. O'Brien. 897-902. Academic Press, New York.

Lonneman, W.A., T.A. Bellar, A.P. Altshuller. 1968. Aromatic hydrocarbons in the atmosphere of the Los Angeles basin. Environ. Sci. Technol. 2:1017.

Ludwig, V.H., A. Werthemann. 1962. Benzol-myelopathien. Schweiz. Med. Wochenschr. 92:378-384.

Lutz, W.K., C. Schlatter. 1977. Mechanism of the carcinogenic action of benzene: Irreversible binding to rat liver DNA. Chem.-Biol. Interact. 18:241-245.

Mallein, M.L., F.A. Byron, D. Fiere, R. Girard. 1971. Crypto leucose aigue ou "anemie refractaire" benzenique (Deux nouveaux cas). Arch-Mal. Prof. 32:577-579.

Mallory, T.B., E.A. Gall, W.J. Brickley. 1939. Chronic exposure to benzene (benzol). III. The pathologic results. J. Ind. Hyg. 21:355.

Maltoni, C., C. Scarnato. 1979. First experimental demonstration of the carcinogenic effects of benzene: long-term bioassays on Sprague-Dawley rats by oral administration. Med. Lav. 70:352-357.

Mara, S.J., S.S. Lee. 1978. Assessment of human exposures to atmospheric benzene. U.S. Environmental Protection Agency, Research Triangle Park, N.C. EPA 450/378-031.

McMichael, A.J., R. Spirtas, L.L. Kupper. 1974. An epidemiological study of mortality within a cohort of rubber workers. Jour. Occup. Med. 15:488.

McMichael, A.J., R. Spirtas, L.L. Kupper, J.F. Gamble. 1975. Solvent exposure and leukemia among rubber workers. An epidemiological study. Jour. Occup. Med. 17:234.

McMichael, A.J., D.A. Andjelkovic, H.A. Tyroler. 1976a. Cancer mortality among rubber workers: An epidemiological study. Ann. N.Y. Acad. Sci. 271:125.

McMichael, A.J., R. Spirtas, J.F. Gamble, P.M. Tousey. 1976b. Mortality among rubber workers: Relationship to specific jobs. Jour. Occup. Med. 18:178.

Meigh, D.F., A. Arthur, E. Filmer, R. Self. 1972. Growth-inhibitory volatile aromatic compounds produced by Solanum tuberosum tubers. Phytochemistry. 12:987-992.

Meyne, J., M.S. Legator. 1980. Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. Environ. Mut. 2:43-50.

Miller, J.A., E.C. Miller. 1977. The concept of reactive electrophilic metabolities in chemical carcinogenesis: Recent results with aromatic amines, safrole and aflatoxin B_1, in Biological Reactive Intermediates: Formation, Toxicity and Inactivation, Jollow, D.J., J.J. Kocsis, R. Snyder, H. Vainio (eds.) 60-24. Plenum Press, New York.

Miller, J.R. 1962. A strain difference in response to the teratogenic effect of maternal fasting in the house mouse. Can. J. Genet. Cytol. 4:69-78.

Moeschlin, S., B. Speck. 1967. Experimental studies on the mechanism of action of benzene on the bone marrow (radioautographic studies using ^{3}H thymidine). Acta. Harmat. 38:104.

Monson, R.R. and K.K. Nakano. 1976a. Mortality among rubber workers. I. White male union employees in Akron, Ohio. Am. Jour. Epidemiol. 103:284.

Monson, R.R. and K.K. Nakano. 1976b. Mortality among rubber workers. II. Other employees. Am. Jour. Epidemiol. 103:297.

Monson, R.A. and L.J. Fine. 1978. Cancer mortality and morbidity among rubber workers. J. Natl. Cancer Inst. 61:4:1047-1053.

Moses, R.E. 1974. DNA synthesis in toluene treated cells of Escherichia coli. In: DNA Replication, Wickner, R.B. (ed.) p. 135.

Murray, J., A. John, L.W. Rampy, R.A. Kuna, B.A. Schwetz. 1979. Embryotoxicity of inhaled benzene in mice and rabbits. Amer. Ind. Hyg. Assoc. J. 40:933.

National Academy of Sciences. 1976. Health effects of benzene: A review. Committee on Toxicology, Assembly of Life Sciences, National Research Council. Washington, D.C.

National Academy of Sciences. 1977. Drinking water and health. Safe Drinking Water Committee, Advisory Center on Toxicology, Assembly of Life Sciences, National Research Council, Washington, D.C.

National Cancer Institute. 1977. On occurrence, metabolism, and toxicity including reported carcinogenicity of benzene. Summary rep. Washington, D.C.

National Institute of Occupational Safety and Health. 1974. Criteria for a recommended standard. . .occupational exposure to benzene. U.S. Department of Health, Education and Welfare, Washington, D.C.

National Institute of Occupational Safety and Health. 1977. Revised recommendation for an occupational exposure standard for benzene. U.S. DHEW, Washington, D.C.

Nau, C.A., J. Neal, M. Thornton. 1966. C9-C12 fractions obtained from petroleum distillates - An evaluation of their potential toxicity. Arch Environ. Health 12:382.

Nawrot, P.S., R.E. Staples. 1979. Teratology. 19:41.

Nencki, M., P. Giacosa. 1880. Uber die oxydation der aromeitischen kohlenwasserstaffe in tierkorper. Hoppe-Zeyler Z. 4:325.

Newsome, J.R., V. Norman, C.H. Keith. 1965. Vapor phase analysis of tobacco smoke. Tobacco Science. 9:102-110.

Nicoll, J.W., P.F. Swann, A.W. Pegg. 1975. Effect of dimethylnitrosamine on persistence of methylated guanines in rat liver and kidney DAN. Nature 254:261.

Nomiyama, K. 1962. Studies on the poisoning by benzene and its homologues (6) Oxidation rate of benzene and benzene poisoning. Med. J. Shinshu Univ. 7:41-48.

Nomiyama, K., H. Nomiyama. 1974a. Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene acetone, ethyl acetate and ethyl alcohol. Int. Arch. Arbeitsmed. 32:75-83.

Nomiyama, K., H. Nomiyama. 1974b. Respiratory elimination of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int. Arch. Arbeitsmed. 32:85-91.

Nonaka, M., D.R. Black, E.L. Pippen. 1967. Gas chromatographic and mass spectral analyses of cooked chicken meat volatiles. Jour. Agr. Food Chem. 15:713-717.

Nylander, P., H. Olaffsson, B. Rasmuson, H. Svahlin. 1978. Mutagenic effects of petrol in Drosophila melanogaster. I. Effects of benzene and 1,2,-dichloroethane. Mut. Res. 57:163.

Occupational Safety and Health Administration. 1978. Final environmental impact statement. Benzene. U.S. Department of Labor, Washington, D.C.

Ogui, T., M. Nakadate, S. Odashima. 1976. Rapid and selective induction of erythroleukemia in female donryu rats by continuous oral administration of 1-ethyl-1-nitrosurea. Can. Res. 36:3403.

Ott, H.G., J.C. Townsend, W.A. Fishbeck, R.A. Langer. 1978. Mortality among individuals occupationally exposed to benzene. Arch. Environ. Health. 33:3.

Pagnotto, L.D., H.B. Elkins, H.G. Brugsch, J.E. Walkley. 1961. Industrial benzene exposure from petroleum naphtha. I. Rubber coating industry. Am. Ind. Hyg. Assoc. Jour. 22:417.

Parke, D.V., R.T. Williams. 1953. Studies in detoxication. The metabolism of benzene containing ^{14}C benzene. Biochem. J. 54:231-238.

Parkinson, G.S. 1971. Benzene in motor gasoline-an investigation into possible health hazards on and around filling stations and in normal transport operations. Ann Occup. Hyg. 14:145-157.

Pellizzari, E.D. Research Triangle Institute. 1978. Quantification of chlorinated hydrocarbons in previously collected air samples. U.S. Environmental Protection Agency, Research Triangle Park, N.C. EPA-450/3-78-112.

Perry, R.H., C.H. Chilton. 1973. Chemical engineers' handbook, 5th ed. McGraw-Hill, New York. pp. 13-36 to 13-42.

Petrini, M. 1941. Investigations on acute and subacute poisoning by benzine and benzene. Rass. Med. Ind. 12:435-476.

Picciano, D. 1979. Cytogenic study of workers exposed to benzene. Environ. Res. 19:33-38.

Pollini, G., G.P. Biscaldi, R. Corsico. 1965. L'Azione del benzolo sull'attivita' proliferativa delle cellule emopoietiche embrionarie. Med. Lavoro. 56:738-745.

Pollini, G., R. Colombi. 1964a Il danno cromosomico midollare nell' anemia aplastica benzolica. Med. Lavoro. 55:241.

Pollini, G. and R. Colombi. 1964b. Lymphocyte chromosome damage in benzene blood dyscrasia. Med. Lav. 55:641.

Porteous, J.W., R.T. Williams. 1949a. Studies in detoxication. The metabolism of benzene. The determination of phenol in urine with 2:6-dichloroquinonechloromide. The excretion of phenol glucuronic acid and ethereal sulfate by rabbits receiving benzene and phenol. Observations on the determination of catechol, quinol and muconic acid in urine. Biochem. J. 44:46-55.

Porteous, J.W., R.T. Williams. 1949b. Studies in detoxication. The metabolism of benzene. The isolation of phenol, catechol, quinol and hydroxyquinol from the ethereal sulfate fraction of the urine in rabbits receiving benzene orally. Biochem. J. 44:56-61.

Posner, H.S., C. Mitoma, S. Udenfriend. 1961. Enzymic hydroxylation of aromatic compounds. Further studies of the properties of the microsomal hydroxylating system. Arch. Biochem. Biophy. 94:269-279.

Pulkrabek, P., T. Konoshita, A.M. Jeffery. 1980. Benzene oxide: *In vitro* mutagenic and toxic effects. Proc. 16th Ann. Meeting Amer. Soc. Clin. Oncol. 21:107.

Pushkina, N.V., V.A. Gofmekler, G.N. Klevtsova. 1968. Changes in content of ascorbic acid and nucleic acids produced by benzene and formaldehyde. Bull. Exp. Biol. Med. 66:868.

Redmond, C.K., A. Ciocco, J.W. Lloyd, H.W. Rush. 1972. Long-term mortality study of steelworkers. II. Mortality from malignant neoplasms among coke oven workers. Jour. Occup. Med. 14:621.

Rickert, D.E., T.S. Baker, J.S. Bus, C.S. Barrow, R.D. Irons. 1979. Benzene disposition in the rat after exposure by inhalation. Toxicol. Appl. Pharmacol. 49:417-423.

Rinsky, R.A., R.J. Young, A.B. Smith. 1981. Leukemia in Benzene Workers. Am. J. of Ind. Med. 2:217-245.

Robustelli della Cuna, G., A. Favino, G.P. Biscaldi, G. Pollini. 1972. Transformazione in leucemia acuta di un caso di mielopatia involutiva benzolica. Haematologia. 57:65-89.

Rondanelli, E.G., P. Gorini, G. Gerna, E. Magliulo. 1970. Pathology of erythroblastic mitosis in occupational benzenic erythropathy and erythemia. S. Karger, Basel.

Runion, H.E. 1975. Benzene in gasoline. Am. Indust. Hygiene Assn. J. 36:338-350.

Runner, M.N., J.R. Miller. 1956. Congenital deformity in mouse as a consequence of fasting. Anal. Res. 124:437.

Saita, G., E.C. Vigliani. 1962. The action of benzene in inducing leukemia. Med. Lavoro. 55:655.

Saito, F.U. J.J. Kocsis, R. Snyder. 1973. Effect of benzene on hepatatic drug metabolism. Toxicol. Appl. Pharmacol. 26:209-217.

Sakamoto, A., K. Inamori, I. Watanabe, E. Fukizawa. 1957. Ring oxidation of benzene. Phenol. formation. Osaka Daigku Zasshi 9:345-347.

Sammett, D., E.W. Lee, J.J. Kocsis, R. Snyder. 1979. Partial hepatectomy reduces both metabolism and toxicity of benzene. J. Toxicol. Environ. Health. 5:785-792.

Santesson, C.G. 1897. Uber chronische vergiftung mit steinkohlentheerbenzin; vir todesfalle. Arch. Hyg. Berl. 31:336.

Sato, A., T. Nakajima. 1979a. A vital-equilibrium method to evaluate the drugmetabolizing enzyme activity for volatile hydrocarbons. Toxicol. Appl. Pharmacol. 47:41-46.

Sato, A., T. Nakajima. 1979b. Dose-dependent interaction between benzene and toluene in vivo and in vitro. Toxicol. Appl. Pharmacol. 48:249-256.

Sato, T., T. Fukuyama, T. Suzuki, H. Yoshikawa. 1963. 1,2-dihydrol-2-dihydroxybenzene and several other substances in the metabolism of benzene. J. Biochem. (Tokyo) 53:23-27.

Savilahti, M. 1956. Over 100 cases of benzene poisoning in a shoe factory. Arch. Gewerbepathol. Gewerbehyg. 15:147-157.

Schofield, K. 1974. Problems with flame ionization detectors in automotive exhaust hydrocarbon measurement. Environ. Sci. Technol. 8(9):826-834.

Schormuller, J. and H.J. Kochmann. 1969. Analyses of volatile aromatic substances in tomatoes by gas chromatography and mass spectroscopy. Z. Lebensm, Unters. Forsch. 141:1-9.

Schrenk, H.H., W.P. Yant, S.J. Pearce, F.A. Patty, R.R. Sayers. 1941. Absorption, distribution and elimination of benzene by body tissues and fluids of dogs exposed to benzene vapor. J. Ind. Hyg. Toxicol. 23:20-34.

Schultzen, O., B. Naunyn. 1867. Uber das Verhalten des Kohlenwasserstoffes im Organismus. Arch. Anat. Phsiol. 349.

Selling, L. 1910. Preliminary report of some cases of purpura haemorrhagica due to benzol poisoning. Bull. Johns Hopkins Hosp. 21:33-36.

Selling, L. 1916. Benzol as a leukotoxin. Studies on the degeneration and regeneration of the blood and haematopoietic organs. Johns Hopkins Hosp. Reports 17:83-142.

Sellyei, M., E. Keleman. 1971. Chromosome study in a case of granulocytic leukemia with "pelgerisation" 7 years after benzene pancytopenia. Europ. J. Cancer 7:83.

Shay, J., M.G. Gruenstein, H.E. Marx, L. Glazer. 1951. The development of lymphatic and myelogenous leukemia in wistar rats following gastric instillation of methylcholanthrene. Can. Res. 11:29.

Sherwood, R.J. 1972. Benzene: The interpretation of monitoring results. Ann. Occup. Hyg. 15:409-421.

Sherwood, R.J., F.W.G. Carter. 1970. The measurement of occupational exposure to benzene vapour. Ann. Occup. Hyg. 13:125-146.

Simonds, J.P., H.M. Jones. 1915. The effect of injections of benzol upon the production of antibodies. J. Med. Res. 33:197-209.

Smith, R.T. 1972. Possibilities and problems of immunologic intervention in cancer. New Eng. J. Med. 287:439.

Smith, R.T. 1970. Cell membrane receptors, in *Immune Surveillance*, (Smith, R.T. and Landy, M., eds.), Academic Press, New York, p. 1.

Smolik, R., K. Grzybek-Hryncewica, A. Lange, W. Zaconski. 1973. Serum complement level in workers exposed to benzene, toluene and xylene. Int. Arch. Arbeitsmed. 31:243.

Snyder, C.A., B.D. Goldstein, A.R. Sellakumar, I. Bromberg, S. Laskin, R.E. Albert. 1980. The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. Toxicol. Appl. Pharmacol. 54:323-331.

Snyder, C.A., B.D. Goldstein, A.R. Sellakumar, S.R. Wolman, I. Bromberg, M.N. Erlichman, S. Laskin. 1978. Hematoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health. 4:605-618.

Snyder, R., J.J. Kocsis. 1975. Current concepts of chronic benzene toxicity. Crit. Rev. Toxicol. 3:265-288.

Snyder, R., E.W. Lee, J.J. Kocsis, C.M. Witmer. 1977. Bone marrow depressant and leukogenic actions of benzene. Life - Sciences 21:1709-1722.

Snyder, R., E.W. Lee, J.J. Kocsis. 1978. Binding of labeled benzene metabolites to mouse liver and bone marrow. Res. Commun. Chem. Pathol. Pharmacol. 20:191-194.

Snyder, R., F. Uzuki, L. Gonasun, E. Bromfeld, A. Wells. 1967. The metabolism of benzene *in vitro*. Toxicol. Appl. Pharmacol. 11:346-360.

Srbova, J., J. Teisinger, S. Skramovsky. 1950. Absorption and elimination of inhaled benzene in man. Arch. Ind. Hyg. 2:1-8.

Steinberg, B. 1949. Bone marrow regeneration in experimental benzene intoxication. Blood 4:550.

Supreme Court of the United States. Argued October 10, 1979, decided July 2, 1980. Syllabus. Industrial Union Department, *AFL-CIO v. American Petroleum Institute, et al*. p. 21-22.

Svirbely, J.L., R.C. Dunn, W.F. von Oettingen. 1944. The chronic toxicity of moderate concentrations of benzene and of mixtures of benzene and its homologues for rats and dogs. J. Ind. Hyg. Toxicol. 26:37.

Tabershaw, I.R., S.H. Lamm. 1977. Benzene and leukemia. The Lancet, October 22, 1977.

Tareeff, E.M., N.M. Kontchalovskaya, L.A. Zorina. 1963. Benzene leukemias. Acta Un. Int. Contra Cancru. 19:751.

Teranishi, R., J.W. Corse, W.H. McFadden, D.R. Black, A.I. Morgan, Jr. 1963. Volatiles from strawberries. I. Mass spectral identification of the more volatile components. Jour. Food Sci. 28:478-483.

Teisinger, J., V. Jiserova-Bergerova, J. Kudrna. 1952. The metabolism of benzene in man. Pracov. Lek. 4:175-188.

Thrope, J.J. 1974. Epidemiological survey of leukemia in persons potentially exposed to benzene. Jour. Occup. Med. 16:375.

Tice, R.R., D.L. Costa, R.T. Drew. 1980. Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. Proc. Natl. Acad. Sci. USA 77:21482152B.

Timbrell, J.A., J.R. Mitchell. 1977. Toxicity-related changes in benzene metabolism *in vivo*. Xenobiotica 7:415-423.

Tough, I.M., W.M. Court-Brown. 1965. Chromosome aberrations and exposure to ambient benzene. Lancet. 1:684.

Tough, I.M., P.G. Smith, W.M. Court-Brown, D.G. Harnden. 1970. Chromosome studies on workers exposed to atmospheric benzene: The possible influence of age. Europ. J. Cancer 6:49.

Townsend, J.C., M.G. Ott, W.A. Fishbeck. 1978. Health exams findings among individuals occupationally exposed to benzene. J. Occup. Med. 20:8:542-548.

Truhaut, R., R. Murray. 1978. International Workshop on Toxicology of benzene, Paris: 9th-11 November, 1976. Int. Arch. Arbeitsmed. 41:65-76.

Tunek, A., F. Oesch. 1979. Unique behavior of benzene mono-oxygenase: Activation by detergent and different properties of benzen- and phenobartibal-induced mono-oxygenase activities. Biochem. Pharmacol. 28:3425-3429.

Tunek, A., K.L. Platt, P. Bentley, F. Oesch. 1978. Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and effects of modification of this metabolism. Mol. Pharmacol. 14:920-929.

Tunek, A., K.L. Platt, M. Przybylski, F. Oesch. (in press). Multistep metabolic activation of benzene. Effect of superoxide dismutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and filed desorption mass spectrometry.

Tunek, A., C. Schelin, B. Jergil. 1979. Microsomal target proteins of metabolically activated aromatic hydrocarbons. Chem.-Biol. Interactions. 27:133-144.

U.S. Environmental Protection Agency. 1978. Assessment of health effects of benzene germane to low-level exposure. U.S. Environ. Prot. Agency, Washington, D.C. EPA600/1-78-061.

U.S. Environmental Protection Agency. 1979. Carcinogen assessment group's final report on population risk to ambient benzene exposures. Office of Air Quality, Research Triangle Park, NC. EPA-450/5-80-004.

U.S. Environmental Protection Agency. 1980. Ambient water quality criteria for benzene. Office of Water Regulations and Standards Criteria and Standards Division. Washington, D.C. EPA 440/5-80-018.

U.S. Environmental Protection Agency. 1972. Industrial pollution of the lower Mississippi River in Louisiana. Region VI, Dallas, Texas, Surveillance and Analysis Division.

U.S. Environmental Protection Agency. 1975. Preliminary assessment of suspected carcinogens in drinking water. Washington, D.C., pp. 9-11 and p. I-13.

U.S. International Trade Commission. 1979. Synthetic organic chemicals. United States production and sales, 1978. U.S. Government Printing Office, Washington, D.C. USITC 1001.

Uyeki, E.M., A.E. Ashkar, D.W. Shoeman, T.U. Bisel. 1977. Acute toxicity of benzene inhalation to hemopoietic precursor cells. Toxicol. Appl. Pharmacol. 40:49-57.

Vianna, N.O., A. Polan. 1979. Lymphomas and occupational benzene exposure. Lancet. 1:1394.

Vigliani, E.C., A. Forni. 1976. Benzene and leukemia. Environ. Res. 11:122.

Vigliani, E.C., G. Saita. 1964. Benzene and leukemia. New England Jour. Med. 172:872.

Vogel, K., P. Bentley, K.L. Platt, F. Oesch. (in press). Rat liver cytoplasmic dihydrodiol dehydrogenase: Purification to apparent homogeneity and properties.

Von Sydow, E. and G. Karlsson. 1971. The aroma of black currants. IV. The influence of heat measured by instrumental methods. Lebensm. Wiss. Techn. 4:54.

Vozovaya, M.A. 1976. The effect of small concentrations of benzene and dichloroethane separately and combined on the reproductive function of animals. G. Sanit. 6:100.

Vozovaya, M.A. 1975. Action of low concentrations of benzene, dichloroethane, and their combination on the generative function of animals and the development of progeny. G. Tr. Prox. Label. 7:20.

Waldradt, J.P., A.O. Pittet, T.E. Kinlin, R. Muralidhora, A. Sanderson. 1971. Volatile components of roasted peanuts. Jour. Agr. Food Chem. 19:972-979.

Walker, P. 1976. Air pollution assessment of benzene. Technical Report. Mitre Corporation, McLean, VA. MTR7188.

Ward, J.M., J.H. Weisburger, R.S. Yamamoto, T. Benjamin, C.A. Brown, E.K. Weisburger. 1975. Long-term effect of benzene in C57BL/6N mice. Arch. Environ. Health. 30:22-25.

Watanabe, G., S. Yoshida. 1970. The teratogenic effect of benzene in pregnant mice. Acta Medica et Biologica. 17:285-291.

Weast, R.C., C.D. Hodgeman, S.M. Selby. 1965. Handbook of chemistry and physics, 46nd ed. The Chemical Rubber Publishing Co., Cleveland.

Weiskotten, H.G., C.B.F. Gibbs, E.O. Boggs, E.R. Templeton. 1920. The action of benzol. VI. Benzol vapor leukopenia (rabbit). J. Med. Res. 41:425-438.

White, W.C., A.M. Gammon. 1914. The influence of benzol inhalation on experimental pulmonary tuberculosis in rabbits. Trans. Assoc. Amer. Phys. 29:332-337.

Wierda, D., R.D. Irons, W.F. Greenlee. 1981. Immunotoxicity in C57BL/6 mice exposed to benzene and Aroclor 1254. Toxicol. Appl. Pharmacol. 60:410-417.

Wildman, J.M., M.L. Freedman, J. Rosman, B.D. Goldstein. 1976. Res. Commun. Chem. Pathol. Pharmacol. 13:473-488.

Wilkens, W.F. and F.M. Lin. 1970. Gas chromatographic and mass spectral analyses of soybean milk volatiles. Jour. Agric. Food Chem. 18:333-336.

Wilson, R.H. 1942. Benzene poisoning in industry. J. Lab. and Clin. Med. 27:1517.

Winternitz, M.C., A.D. Hirschfelder. 1913. Studies upon experimental pneumonia in rabbits: Parts I-III, J. Exptl. Med. 17:664.

Wolf, M.A., V.K. Rowe, D.D. McCollister, R.L. Hollingsworth, F. Oyen. 1956. Toxicological studies of certain alkylated benzenes and benzene. Arch. Ind. Health 14:387-398.

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Wolman, S.R. 1977. Cytologic and cytogenetic effects of benzene. Toxicol. Environ. Health Suppl. 2:63.

Wong, N.P., et al. 1967. Decomposition and filth in foods. Investigation of volatile components in cod fish by gas chromatography and mass spectroscopy. Jour. Assoc. Off. Anal. Chem. 50:8.

Yant, W.P., H.H. Schrenk, R.R. Sayers, A.A. Howarth, W.A. Reinhart. 1936. Urine sulfate determination as a measure of benzene exposure. J. Ind. Hyg. Toxicol. 18:69.

Young, R.J., R.A. Rinsky, D.F. Infante. 1978. Benzene in consumer products. Science 199:248.

Zenz, C. 1978. Benzene attempts to establish a lower exposure standard in the United States. A review. Scand. J. Work. Environ. and Health. 4:103-113.